LOCAL IMMUNIZATION

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SPECIFIC DRESSINGS

BY

PROFESSOR A. BESREDKA

PASTEUR INSTITUTE, PARIS

EDITED AND TRANSLATED BY

DR. HARRY PLOTZ

PASTEUR INSTITUTE, PARIS

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TO THE HONORED MEMORY OF

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PREFACE

The object in publishing this small volume, is to present to those interested in the problem of immunity, a point of view which is different from the one that has been followed up to the present.

The idea of local immunity as we conceive it, that is, an immunity without the obligatory participation of antibodies, has barely made its appearance.

This conception already rests upon a large number of facts. Many of the phenomena, which can not be explained by the accepted theories, are cleared up in the light of this new conception. As a result of these researches, applications to vaccination and vaccinotherapy have followed, and are now being employed in daily practice.

It is not our purpose here to unite all the facts pertaining to local immunization. In order to shorten this volume we have omitted the subjects of cholera infection and vaccination, foot and mouth disease, vaccinia and smallpox vaccination, and some others. To make our main idea clear, we have chosen a number of type infections, which deal with diseases of the skin and the intestine. The four chapters that are devoted to them contain laboratory experiments, and, from time to time epidemiological and clinical observations.

The fifth chapter, which is the last, is devoted to theoretical considerations, or, to employ the phrase of Pasteur,[1] to "the illusions of the experimentor, which serve as a guide, and which gradually fade as we travel along the road."

In it we will find the theory of local immunity, as the

[1] Letter to Blanchard. C. R. Acad. Sciences, Vol. XI, 1880.

truth appears to us today. For the present, it stimulates work and discussion. This is its merit. We are far from believing that it will stand forever—it will surely go the way of its predecessors. But is this important? Theories disappear, but the facts remain. As transitory as it may be, the theory is based on reason. Was it not while speaking of preconceived ideas in biology, that Claude Bernard, the creator of the experimental method affirmed, that it was ideas that edified science, which constituted the *primum movens* of all reasoning, and therefore satisfied the aspirations of the mind toward the unknown?[2]

[2] Introduction a l'étude de la médecine experimentale.

A. BESREDKA.

TRANSLATOR'S PREFACE

It has been my pleasure to be associated with Professor Besredka at the Pasteur Institute for the past five years. During this period, a great deal of the work on local immunization was developed. The subject has opened up new fields of investigation and practical application, and for that reason, I thought that an English translation would be of interest to English speaking bacteriologists.

In this translation, I have tried to convey the author's thoughts as faithfully as possible. In a few instances, I have deviated from the text, and in the second chapter, new cases have been substituted and added. All of these changes however, have received the approval of the author.

HARRY PLOTZ.

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CHAPTER I

ANTHRAX

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c. Application of the principle of cutaneous vaccination to large animals· Experiments on horses by Brocq-Rousseu and Urbain. Nicolas's studies in the army of the East. Experiment on sheep by Velu. General conclusions.

I

Let our thoughts carry us back seventy-five years to the time of the well-known biologist Rayer, who being moved by the damage caused by the disease "sang de rate"[1] at Beauce,[2] took the initiative to call together the doctors and veterinarians in the department of the Eure and Loire,[3] in order to organize a plan for combating "anthrax diseases." At the period referred to, this was the manner in which epidemics amongst animals were named, which for years had caused such great loss to the agriculturists.

[1] Literally, "blood of the spleen"—term applied to anthrax infection.

[2] Beauce—just south of Paris. One of the granaries of France.

[3] One of the 86 departments. Southwest of Paris.

The public officials, not only in France, but in other countries, were greatly concerned with this problem. There were few regions in the world that did not suffer. The annual loss totaled hundreds of millions of francs.

The malignant pustule in man also claimed its victims. It was known however that it followed penetration of the skin with anthrax infected blood.

All were of the opinion, that as long as the method of dissemination of the infection was not known, it would be impossible to combat the "black disease." This unanimity of opinion however ceased, when they discussed the nature of the virus or the factors which favored its dissemination. While one group believed that it was a contagious disease, the other, a much larger one, refused to accept this. The latter credited various conditions as being responsible for it, such as the nature of the soil, mouldy fodder, overeating, or inanition, too uniform a diet, or atmospheric conditions. Since the disease could be transmitted at a distance, without direct contact between sick and healthy animals, some considered the possibility of a volatile virus. Others however, incriminated invisible animalculi or ferments—without much conviction, to be sure.

These divergent points of view did not aid the interested individuals in determining upon a plan for combating the disease. For this reason we see the opinion gradually gain favor—an opinion that the medical men of the period readily held—that the disease depended upon "the plethoric, congestive or apoplectic state of the animals." Starting with this point of view, the practitioners decided to relieve the animals of this congestion by extensive bleedings, and at the same time, neglected to take the most elementary prophylactic precautions.

It was during this period of general disorganization—that is, the period when the idea of contagion was regarded

with hostility—that the bacterial origin of anthrax took form. The department of the Eure and Loire had the honor of being the birth place of the anthrax bacillus—an organism which was the first of the pathogenic bacteria.

In 1850 Rayer took with him his young assistant, C. J. Davaine to Chartres[4] where the former presided at a meeting of the commission. With the mind of an observer and investigator, Davaine immediately concentrated his attention on the study of the blood. On examining sheep dead of anthrax, he quickly observed the peculiar distribution of the red blood cells. Instead of being separated, as occurs in normal blood, he found that the cells appeared in small irregular agglutinated masses. Another fact which struck him as being of importance, was the presence of small filiform non-motile bodies in the blood, which were about twice the length of a red blood cell. Very timidly Davaine expressed the opinion that these small bodies might not be foreign to the causative agent of the disease. What audacity this young assistant must have had to think that a highly developed animal organism, as complex as the sheep, could be invaded and even destroyed by such a small "vibrion," as it was called in 1850!

The first communication by Davaine on the subject, presented before the Society of Biology, appeared in the eyes of his contemporaries as of little importance—no discussion was aroused, and soon after, it was forgotten.

Davaine only returned to his idea much later, being stimulated by Pasteur's work on the butyric ferment. Even though the minds of his contemporaries were better prepared, the young scientist was still subjected to numerous and severe criticisms.

[4] Southwest of Paris. The seat of the beautiful thirteenth century Gothic cathedral.

At the time however, no one could ignore the presence of these "vibrions" in animals dead of the disease. But no one saw any relationship between them and the cause of the infection. The "vibrions" were regarded as being derived from post-mortem decomposition, or as accidental findings, without any relationship to the infection. His critics declared that "they do not appear before death," or in other words, that they appeared only after death, and hence do not exist in the animal during the disease.

The opinions held by Davaine were most unpopular with the veterinarians. One may judge of this by what Delafond, one of the most distinguished teachers at the school at Alfort,[5] said. "I am far from believing that these bodies which breed in the disease, and the real virus which transmits the disease, are the same." This was written in 1860, or ten years after Davaine's discovery.

Davaine held to his opinion, in spite of the skepticism of the older scientists. He presented a series of communications before the Academy of Sciences, in which he firmly defended the importance of these "filiform infusoria," and their probable importance in the disease called "Sang de rate." In 1863 he called these bodies "bacteridie"[6]—a name which still remains.

One of his communications is worthy of mention here. It was entitled, "Experiments to prove that these bacteria constitute the only virus in anthrax." It is interesting, for it takes us back to the older methods, and so permits us to measure the road traveled in bacteriological technic. This in short, was the method he employed.

A few drops of dried anthrax blood was diluted in 50 cc. of distilled water, which was contained in a narrow flask. After 24 hours, the greatest number of bacteria

[5] Government School for Veterinarians

[6] Anthrax bacillus.

settled at the bottom of the flask, while very few remained in the upper layer. In order to avoid putrefaction, about half of the water of the upper part was replaced with fresh water;—this procedure was repeated many times. The liquid remained perfectly clear, except the lower layer which became slightly cloudy. When one drop of the upper liquid was inoculated into a guinea-pig, nothing happened; but one drop of the liquid from the lower part, inoculated into another guinea-pig, induced a fatal anthrax infection.

This was the experiment that proved that "the disease is not in us, from us, and by us," as was believed at the time; but that it was caused by an agent that is foreign to the body.

Even though this demonstration is not absolutely convincing to us to-day, still the technic devised by Davaine, is of interest to those who wish to trace things back to their origin.

In spite of the fact that Davaine multiplied his demonstrations, he could not convince his adversaries.

"Anthrax is not a parasitic disease," declared Jaillard and Leplat, "for the parasite can not be found in the blood of infected animals." "The bacterium is only an accident of the disease." These were the conclusions of the two professors at Val-de-Grace.[7]

Davaine repeated his experiments, but this time conducted them in the same manner as his critics. As a matter of fact he was unable to demonstrate the presence of the bacteria in the blood. "It can not be otherwise" maliciously added Davaine, for the disease taken to be anthrax by Jaillard and Leplat was an entirely different condition. The reason for this is as follows. An animal

[7] Military Medical School.

dead of anthrax, when abandoned, undergoes putrefaction. The Vibrion septic appears and masks the presence of the anthrax bacillus. The latter bacterium finally disappears from the blood and a new infection replaces the anthrax. It was this confusion of the two conditions that obscured for a number of years the nature of the diseases then called anthrax.

Davaine's critics did not consider themselves beaten. We need only recall the criticism of Colin, "the great believer in the spontaneity of all diseases," as Pasteur characterized him. Let us also recall the objections raised by Signol, who in 1875 in a communication before the Academy of Sciences said, that it was only necessary to asphyxiate a healthy animal in order to render its blood virulent and to be able to find bacteria in it which were identical to those found in anthrax.

How greatly impressed they must have been after the announcement of the following experiment, which brought forth a great deal of discussion.

"I can kill the bacteria in a drop of dried blood with compressed oxygen, inoculate the remaining part and produce the disease and death, without ever being able to demonstrate the presence of the bacterium. Therefore, the bacteria are neither the cause nor the effect of anthrax."

These lines appeared 27 years after Davaine's discovery. They carried great weight, for they were written by Paul Bert, one of the best known investigators of his time.

These conclusions, as was later shown, were due to an error in interpretation. Paul Bert did not know of the spores that Robert Koch had described in his well-known communication in 1876. All of Pasteur's authority was needed in order to stop this violent controversy. This intervention was important for Davaine, for all his critics ceased their attacks as if under a spell.

A great deal of research and of active discussion were therefore necessary, in order to prove that the disease "Sang de rate" was not due to witchcraft, nor to a charm, as the agriculturists believed; nor that it was caused either by a volatile virus or an animalculi, as the veterinarians held; but simply to a microscopic plant. More than a quarter of a century of effort and active discussion were necessary, in order to establish a truth—which today requires an instant to convince even the least initiated.

This page of history just recalled, is so intimately connected with Davaine, that it may be of interest to record some of the personal traits, that characterized this fine figure. This great discoverer, who served bacteriology so well, was very modest. He sought neither position nor honors, having no other ambition than to do good and be of service. During many years, if not during his entire lifetime, he was faced by a general opposition. It was only at the end of his career, that he saw his opinions triumph. The prediction of d'Andral that, "bacteria will carry Davaine to the Institute"—the dream of all French Scientists—did not materialize. In spite of the assistance of Pasteur, he was not admitted. He died in 1882, at the age of 70.[8]

The discovery made by Davaine pointed to a new means of combating anthrax. The workers were still uncertain however, of the conditions that brought about the spread of the disease. To be sure, it was of great importance to know the germ that had to be combated; but this alone was not sufficient. It was important to determine the source of the virus and how it was transmitted. These two epidemiological problems were solved, as we know, by the experiments of Koch and Pasteur. Let us recall them briefly.

[8] Laboulbene, Report on C. J. Davaine, 1884.

Pasteur wondered whether the reservoir of the virus might not be the dead anthrax animals that had been buried in the ground. With this hypothesis he turned directly to his experiments. After sprinkling a quantity of soil with yeast water and urine, he added anthrax infected blood. Sometime later, he noticed that the bacteria multiplied and finally developed into "corpuscular-germs." This soil, which was abandoned for some months, was subsequently re-examined by Pasteur. He found spores ready to germinate, and capable of inducing a fatal anthrax infection.

At the same time that this laboratory experiment was being conducted, Pasteur made another under conditions that more closely resembled those found in nature.

A sheep that had died of an anthrax infection was buried in the garden of a farm. Pasteur found that the bacteria remained alive and virulent in the ground two years later. Of great importance was the observation, that even though the ground is a good filter, a large quantity of bacteria was found in the upper layer of the soil. The problem that presented itself was, what made the bacteria leave the deeper layers in which they were interred, to come up to the upper strata?

Pasteur immediately thought of the worms in the earth. He surmised that it must be these worms that carried the bacteria to the surface of the soil.

He must have reasoned, that this migration was not devoid of serious consequences. For the excreta of the worms dry and become dust on reaching the air—the dust spreads to the plants and so soils the crops—the animals that come to graze, naturally ingest the virus, and in this manner contract anthrax. In this way the "corpuscles" remaining on the grazing ground are the agents that propagate the epidemics.

Was this chain of reasoning simply a fancy? Not at all. It was not difficult for Pasteur to prove that sheep could be given anthrax by permitting them to eat clover soiled with bacteria. This experiment succeeded particularly well, when the food was mixed with sharp objects—such as the pointed ends of dried thistle leaves or small pieces of the ears of bearded barley. We will subsequently see how the experiment made by Pasteur clarified the mechanism of experimental infection. At present let us recapitulate the facts relative to natural infection.

The anthrax virus lies in the ground where the infected cadavers are buried; this ground becomes enriched with anthrax spores; these spores are carried to the surface of the soil by the worms, and are then spread to the crops; the crops are ingested by the animals, and finally they contract the disease.

The worms alone are not the only means of spreading the spores from the deeper layers to the surface of the soil. There are certain insects living in the ground, that come to the surface from time to time for air, and hence they may do the same thing. Ploughing up of the soil may also aid the bacteria in the deeper layers to reach the surface, and so infect the crops.

The propagation of anthrax from infected cadavers now being established, the prophylaxis seemed to be an easy matter. There was only one precaution necessary in order to prevent epidemics, and that was to bury the animals far away from the fields where the crops were gathered or where the sheep grazed.

Is infection in nature produced in any other manner than through the digestive tract?

Long before the researches of Pasteur, Davaine drew attention to the possibility of contamination by way of the

skin. He was led to regard the skin as a portal of entry of anthrax infection, while studying the malignant pustule in man.

A curious experiment, quite forgotten now, should according to Davaine prove the origin of the malignant pustule in man and the "Sang de rate" in animals.

A cautery that had been heated in boiling water was applied to the skin of a guinea-pig. As soon as the vesicles appeared he introduced a small quantity of anthrax blood, taking care not to break the vesicle. Nothing appeared the first day, but on the next the vesicle gradually became larger. It was surrounded by a red area, and was filled with anthrax bacilli.

This then was the experimental production of the malignant pustule in the guinea-pig, and *eo ipso* the proof of the possibility of inoculating anthrax, by way of the skin.

Considering the very small amount of anthrax blood necessary to produce infection in man, Davaine thought that perhaps a fly-bite would be sufficient to produce a malignant pustule. Following this he was led to believe that also in animals the fly might play an important rôle in transmitting infection to them; the spineless fly by depositing infected material on skin lesions; the biting fly by carrying blood from an infected to a healthy animal.

Even though the rôle of the fly can not be demonstrated with certainty in the animal, initial infection of the skin in man could not be questioned. Davaine studied the histology of the pustules carefully and recorded the distribution of the bacteria in the various layers of the skin. In a series of communications, presented before the Academy of Sciences between 1872 and 1881, he showed that the malignant pustule in man is always superficial at the start; that it begins under the epidermis in the mucosa,

entirely cut off from the circulation; that the bacteria subsequently invade the subcutaneous tissue and produce an edema; that it is only rather late that the bacteria enter the circulation and the internal organs, and so the disease becomes generalized.

To summarize: In the ordinary method of infection the bacteria attack the animal either by way of the digestive tract or by way of the skin. It is now more than 40 years since the cutaneous infection of Davaine, and especially the entero-infection of Pasteur, have been established.

"I dare hope that anthrax will soon become to us all a problem more simple than favus—a disease that science will explain, that hygiene will render more and more rare, and even possibly make it disappear from all those places where it has caused devastation since the beginning of the century." These prognostications, formulated by Davaine in 1870 were in part realized ten years later as the result of Pasteur's researches.

As a result of his work on chicken cholera, Pasteur discovered the principle of the attenuation of the virus. He found that a chicken, inoculated with an attenuated chicken cholera virus, could be protected against a severe or fatal infection caused by the inoculation of a non-attenuated virus. Pasteur naturally wondered whether the same might not hold true for anthrax. It should he reasoned, a priori, if anthrax is a disease that does not recur in recovered animals.

Pasteur was soon able to make an observation of great importance which bought him the information he desired. A cow which fell ill, following an inoculation with the virus of anthrax, developed an edema and a sharp rise of temperature. The infection then subsided. This then afforded an excellent opportunity to solve the problem of the possibility of re-infection. As a matter of fact, when

this cow was subsequently inoculated with virulent virus it remained refractory.

"By virtue of my communications pertaining to chicken cholera, we know of a virulent parasitic disease which is incapable of re-infection. We now have a second example in anthrax infection. We know that in anthrax, as in chicken cholera, that the inoculations which do not kill, are preventative."

The idea of non-reinfection being established, Pasteur tried to find a practical method which would permit him to attenuate the bacterium at will, so as to create a benign infection which would protect the animal against a fatal anthrax infection.

We know how he was able to create a series of cultures with a determined virulence, after having started with a virulent strain. "Now it should be very easy to see whether these various viruses can be given to sheep, cows and horses, without giving them anthrax, and at the same time protecting them against a subsequent fatal infection."

The memorable experiments at Pouilly-le-Fort[9] justified Pasteur's prediction in a remarkable manner. On May 5, 1881, at a farm now called "Clos Pasteur"—24 sheep, a goat and 6 cows received five drops of an attenuated anthrax culture by subcutaneous injection. On May 17 the 24 sheep, the goat and the 6 cows received a second subcutaneous injection of an attenuated culture, but one that was more virulent than the first. On May 31 the degree of immunity in the 31 vaccinated animals was determined. As controls, Pasteur employed 29 new animals—24 sheep, a goat, and 4 cows. All the animals were inoculated with a very virulent anthrax culture.

[9] Southeast of Paris, near Melun.

Two days later, that is on June 2, a number of people met at Pouilly-le-Fort to see the result of the vaccination.

The visitors were profoundly moved when they saw the 24 vaccinated sheep, and the goat all in good health, while the 21 sheep and the goat not vaccinated, already lay dead on the ground. Two other non-vaccinated sheep died under their eyes, while the remaining animal died at the end of the day.

This experiment marked a turning point in the history, not only of anthax, but in medical science as well. This was the beginning of important discoveries, which continue to increase from year to year.

More than 40 years now separates us from the experiment at Pouilly-et-Fort. Bacteriology has made such advances that we can not find its equal in any other branch of biology. Bacteriology today dominates medicine, surgery and hygiene, to mention only those sciences familiar to us. Workers in all countries have attacked the most varied problems pertaining to bacteria. New chapters have been created, old ones have been completed or begun anew. The chapter on anthrax remained unchanged amidst all these transformations; it remained almost the same as it was handed down to us by Davaine, Koch, Pasteur and his co-workers Roux and Chamberland. Had it not been for the controversy, which sprang up between the partisans of the cellular and humoral theories of immunity at the end of the last century, which created a new interest in this bacterium, the chapter on anthax would have been considered definitely closed.

II

In 1921 a communication appeared in the Annales of the Pasteur Institute[10] which presented the problem of the

[10] Annales de l'Institut Pasteur, July, 1921, 35, p. 421.

mechanism of infection and immunity in anthrax, in a new light.

The title of this article was "cuti-infection (cutaneous infection), cuti-vaccination (cutaneous vaccination), and cuti-immunity (cutaneous immunity)." These three terms created for the communication should, according to the author, summarize the new facts observed.

The article referred to was the basis of all the researches which have since followed on the subject of local immunization. We will therefore repeat here fully the substance of this article.

The classical method of anthrax vaccination succeeds remarkably well in large animals, but fails absolutely in small laboratory animals. The moment we exceed the minimal lethal dose in the guinea-pig, which is 0.1 to 0.01 cc. of the second vaccine,[11] the animal rarely survives. We may previously prepare the guinea-pig over a period of months, graduating this preparation with all possible care, either by subcutaneous, intraperitoneal or intravenous inoculation, but the result is always the same; the guinea-pig can not be vaccinated.

Ever since the beginning of these experiments our attention was drawn to the manner in which the skin reacted to the anthrax vaccines, especially after irritation of the skin. When we rub a swab soaked in the first vaccine on the freshly shaved skin of a guinea-pig, we notice a characteristic inflammatory reaction the next day.[12] There is no tendency to invade the neighboring areas. The reaction continues from 4 to 6 days and then gradually disappears. The skin becomes pale, soft and finally leaves no apparent evidence of the treatment.

The reaction is entirely different when the second vac-

[11] First and second vaccines, refer to the first and second Pasteur vaccines.

cine is applied. The application of this vaccine to the shaved skin of a normal guinea-pig is followed by a severe inflammatory reaction. It is more intense than in the previous instance; instead of remaining localized to the area of application, the reaction extends to the neighboring tissues, spreads further and further, and rapidly becomes generalized. The guinea-pig dies in about 4 days.

These are the characteristics of the skin infection following application to the skin with the first and second anthrax vaccines.

As we have just seen, the guinea-pig that had the shaved skin rubbed with the first vaccine gets well quite rapidly. Is it just a simple return to the normal state? Has some modification taken place in the tissues, that distinguishes it from a normal guinea-pig?

When the skin has returned to normal let us submit this guinea-pig to a new application, but this time employing a more virulent virus than in the first instance. Shave the skin and then apply the second vaccine to any part of the skin which is still raw and red. For comparison the same procedure should be carried out with a normal guinea-pig.

The next day we notice a local reaction, more or less severe, and about the same intensity in the two animals. Following this, the lesion in the control animal continues to spread and rapidly terminates with a fatal septicemia. In the guinea-pig which had previously been treated with the first vaccine, the reaction remains circumscribed. Not only does the infection not spread, but in a few days the skin returns to its normal aspect.

This guinea-pig then has acquired a certain degree of immunity, by virtue of the first application.

[13] The skin of the abdomen is shaved and a swab soaked in vaccine is applied. An area of skin 2 by 4 inches is shaved, so that the skin appears raw and red.

Let us continue the experiment. When all traces of the skin lesion have completely disappeared, let us submit the guinea-pig to a new, but more severe test than on the first instance. Let us apply to the freshly shaved skin a non-attenuated broth culture of anthrax bacilli.

An active reaction follows—the shaved area becomes red, indurated and even may develop some edema; but the reaction stops there. In spite of the dose of virus given—a dose sufficient to kill any animal not previously prepared—the guinea-pig will survive.

In order to illustrate this, we will cite the history of a few guinea-pigs cutaneously vaccinated.

Guinea-pig A. 530 grams.
April 2. First vaccine applied by rubbing[13] into skin.
April 8. Second vaccine applied by rubbing[13] into skin.
(Control died April 12.)
April 23. Second vaccine 0.25 cc. injected subcutaneously.
(Control received 0.125 cc. and died on the 27th.)
May 5. Pure culture virus applied by rubbing on skin.
May 11. Pure culture (0.1 cc.) injected subcutaneously.
(Control died)
Animal survives.

Guinea-pig B. 580 grams.
April 21 First vaccine applied by rubbing on skin.
May 5. Second vaccine applied by rubbing on skin.
May 18. Pure virus applied by rubbing on skin.
May 26. Pure virus (0.1 cc. of broth culture) injected subcutaneously.
June 2. Pure virus 1 cc. injected subcutaneously.
June 15. Pure virus 3 cc. injected subcutaneously.
June 29. Pure virus 5 cc. injected subcutaneously.
July 2. Pure virus 1 cc. of an agar culture injected subcutaneously.
Animal survives.

[13] Skin is shaved and vaccine is rubbed on the shaved area with a swab soaked in the vaccine.

The rabbit may be cutaneously vaccinated against anthrax with the same ease—perhaps even more so than the guinea-pig.

Rabbit, 2050 grams.

April 23. Second vaccine app'ied by friction, on shaved skin.
April 30. Pure virus applied by friction, on shaved skin.
May 11. Pure virus 0.1 cc. of broth culture injected subcutaneously.
(Control died on the 17th.)
May 19. Pure virus 5 cc. of broth culture injected subcutaneously.
June 6. Pure virus 4 cc. of broth culture in trachea.
Animal survives.

An anthrax immunity, quite as solid as that described above, may be obtained by direct inoculation into the skin. Instead of applying the vaccines to the shaved skin, we can succeed just as well by injecting the first and then the second vaccine directly into the thickness of the skin of the guinea-pig. This method has the advantage of enabling us to determine the exact dose injected.

We will cite two examples, in order to give an idea of the local reaction and the course that follows this method of vaccination.

Guinea-pig, 510 grams.

October 28. 1 cc. of first vaccine injected into skin.
October 29. Skin red and edematous.
October 30. Redness and edema increased.
November 1. Skin reddish violet.
November 2. Scab.
November 3. Black scab on a red edematous background.
November 4. Black area about 10 cm. in diameter.
November 5. This area about 5 cm. in diameter.
November 6. Thick black scab resting on a pink background.
November 8. Base is pale
November 9. Scab still adherent.
November 10. Scab begins to loosen at edge.

November 12. On the spot where the scab formerly was attached, is a bleeding ulcerated area.
Cicatrization continues on following days.
December 6. Skin is normal.
December 8. 0.1 cc. of second vaccine injected into the skin.
December 9. Skin is edematous and excoriated in places.
December 10. Thin black scab, slightly adherent.
December 13. The scab, on being detached, exposes a large ulcerated area that is beginning to cicatrize.
December 14. And following days, cicatrization is completed.
December 21. The skin appears normal.
December 24. 0.1 cc. of anthrax virus injected into the skin.
December 25. Skin is red.
December 27. Same.
December 29. Skin normal.
January 4. 1 cc. of anthrax virus injected subcutaneously.
January 6. Large edematous area.
January 10. Well.
Animal survives.

We see then, that a guinea-pig which has received a large dose (1 cc.) of the first vaccine into the skin presents a long and intense local reaction; but this is followed by a solid immunity.

In order to avoid this active reaction at the beginning, we recommend starting the injections with a small dose (0.1 cc.) of the vaccine. If we proceed in this manner, we may create just as solid an immunity in a relatively short time. Here is an example.

Guinea-pig, 490 grams.
November 4. 0.1 cc. of first vaccine injected into the skin.
November 5. Small superficial erosion of skin.
November 6. Small scab, slight edema.
November 9. Same.
November 10. Small black scab.
November 12. Black scab on normal background.
November 13. And following days, scab detached
November 25. Skin normal.

November 27.	0.1 cc. of first vaccine injected into the skin. (Same dose as first time.)
December 4.	Small scab.
December 6.	Skin normal.
December 8.	0.1 cc. of second vaccine injected into skin.
December 9.	Skin slightly indurated.
December 13.	Skin normal.
December 17.	0.1 cc. broth culture of virus injected into the skin.
December 18.	No reaction.
December 24.	0.5 cc. of virus injected into skin.
December 27.	No reaction.
January 4.	1 cc. of virus injected subcutaneously.
January 6.	Edema.
January 10.	Edema disappeared.
February 11.	1 cc. of virus injected into skin.
February 18.	No reaction.
	Exanguinated (see below).

We observe then, that either by rubbing the vaccine into the shaved skin, or by inoculating it directly into the skin, we are certain to confer an immunity upon the guinea-pig, which will enable it to resist any dose of anthrax virus injected into any part of the animal body.

We find therefore in the case of the guinea-pig, that an anthrax immunity, quite unknown up to the present, is easily obtained by vaccinating the skin.

How do we explain the mechanism of this immunity?

The first thought that comes to mind is that the blood of the immunized guinea-pig contains antibodies—which, for some unknown reason—are only induced by skin vaccination.

Even though this idea is open to question, the hypothesis was tested experimentally. Without stopping to search for complement fixation and agglutination—this will be considered later in reference to cutaneously vaccinated horses—we first sought for the presence of any protective

properties in the blood of the cutaneously vaccinated animals.

One of our guinea-pigs, which had acquired a very solid immunity against the virus of anthrax, following cutaneous vaccination was exsanguinated. The serum was injected into two normal guinea-pigs, to test its protective properties, and the following day these animals were inoculated with a fatal dose of virus.

Guinea-pig.	Cutaneously vaccinated (see above) received:
January 4.	1 cc. of anthrax virus injected subcutaneously.
February 11	Same dose of virus injected into the skin. Local reaction slight.
February 18.	Exanguinated. After coagulation and centrifuging, 1 cc. of serum was injected subcutaneously into each of two normal guinea-pigs.
February 19.	One guinea-pig received 0.25 cc. of the second vaccine by subcutaneous inoculation, and the other 0.001 cc. of virus in the same manner. Two controls were inoculated in the same way.

The two prepared guinea-pigs died on February 22 and 23 respectively, with characteristic edema and anthrax bacilli in the blood. The controls died after about the same period of time (February 21 and 22).

It follows from this experiment, that a guinea-pig may be actively vaccinated cutaneously, but its blood does not possess any protective properties. The immunity present in the guinea-pig then does not depend upon the presence of specific antibodies.

Is the immunity acquired in this instance comparable to that obtained with abrin?

The immunity developed in the ocular conjunctiva with abrin, does not extend to the entire animal organism. Not only is the animal organism not protected following this vaccination, but of more importance, the immunity

acquired in the prepared conjunctiva does not extend to the other eye. The latter remains just as sensitive to abrin as the conjunctiva of a normal animal.

Cutaneous vaccination against anthrax on the other hand confers a solid immunity to the entire animal organism. What is the nature of this immunity?

At present this is the point of view we hold. We believe that in the guinea-pig, there is an organ which has a real affinity for the anthrax bacillus, an organ in which this bacterium may be implanted and in which it may multiply and secrete its toxin. This tissue is the skin. Outside of the skin the anthrax bacillus acts as a saprophyte.

But, it may be objected, the guinea-pig dies following an intraperitoneal, intratracheal or subcutaneous inoculation of anthrax virus, quite as it does following an inoculation into the skin. To be sure, a guinea-pig may die of anthrax infection, irrespective of the portal of entry of the virus, but we believe that the animal always dies because of the skin infection. The edema that is found almost always at the site of penetration of the needle, is due we believe, to the fact that during the process of inoculation, the skin was not protected from infection. We injure or involuntarily infect the skin, either while inserting the needle to inoculate the virus, or while withdrawing it. This slight injury which we have caused the animal is fatal—for a guinea-pig which has its skin contaminated with anthrax bacilli may be regarded as a dead animal.

If this hypothesis, which we have just expressed be correct, it demands a further proof which should be easy to establish. For if the skin is protected from infection, the guinea-pig should remain insensitive to an inoculation of the virus. This has been shown to be the case. We will not enter into the delicate technical details of the experi-

ments, which have already been confirmed by others. Let us simply state that a dose of virus, more than 100 times the lethal dose, may be injected into the trachea, the peritoneum, the intestine, the brain or elsewhere, without causing the animal the least harm.

Basing our opinion on these experiments, we may express the idea that the receptivity of the guinea-pig to anthrax, resides principally, if not entirely in the skin. The guinea-pig that has been inoculated with the anthrax virus does not die of a septicemia, as is generally believed, but dies because it develops a cutaneous infection at first, or a cutaneous intoxication; the septicemia occurring later.

In the light of this conception we may see why a guinea-pig that has been cutaneously vaccinated develops such a solid immunity. We also understand why a local immunization conferred only to the skin, carries with it an immunity, which is not only localized, but extends to the entire animal organism. It follows from our experiments on the guinea-pig, that there is only one organ which is sensitive—and that is the cutaneous apparatus. Just as soon as this organ is vaccinated, the entire animal becomes vaccinated; the virus may be introduced into the peritoneum, into the respiratory tract, into the intestinal canal or elsewhere, but the guinea-pig remains unaffected. In this instance the vaccinated skin becomes a barrier to the ingress of the virus, while the other organs show an indifference to it.

It is important to note that the normal guinea-pigs which have survived enormous doses of virus after intraperitoneal, intratracheal or other injections (naturally those animals in which the skin was not infected), show no immunity to a subsequent inoculation of virus. When these

animals are injected subcutaneously with a fatal dose of virus, they die of anthrax quite like the controls. We know that anthrax bacilli which enter by routes other than the skin, do not disturb the animal. The moment they enter the organism they are phagocytosed and digested. This destruction must be rapid and complete, for the animal shows no evidence of having received them.

Immunization or non-immunization of the animal runs parallel with the sensitivity or non-sensitivity towards the virus. Likewise, cutaneous immunity is the response of the organism to the cutaneous infection.

To summarize: The first anthrax vaccine applied to the shaved skin of a guinea-pig or injected into the thickness of the skin, gives rise to a local, self limited lesion. Under the same conditions, the second vaccine in a normal guinea-pig gives rise to a cutaneous reaction which is followed by a fatal septicemia.

A guinea-pig, which has already received the first vaccine into the skin, is not seriously affected by the cutaneous application of the second vaccine. The reaction remains localized at the site of inoculation and finally heals.

An animal which has resisted the second vaccine after application to the skin or after injection into it, survives the inoculation of the same vaccine when inoculated under the skin. The animal will subsequently resist the inoculation of active culture, either by subcutaneous or intracutaneous injection.

The serum of a guinea-pig, cutaneously vaccinated, contains no antibodies capable of protecting a normal guinea-pig against an anthrax infection.

The sensitiveness of a normal guinea-pig to anthrax resides in all probability in its skin. The normal guinea-pig is insensitive to anthrax infection, when the virus is inoculated by any other method than into the skin. The

animal is likewise refractory to all methods of vaccination other than vaccination of the skin.

Cutaneous vaccination confers, in a paradoxical manner, a general immunity upon the entire animal organism. This is due to two factors—to the immunity of the skin acquired by cutaneous vaccination, and to the natural refractory state of all the other organs.

We have seen that the guinea-pig has always been cited as the animal most sensitive to anthrax infection. Likewise the guinea-pig has acquired the reputation of being particularly resistant to anthrax vaccination. The classical remark has been that vaccination of the guinea-pig was difficult, if not impossible,—because of its great susceptibility to the virus.

We now know that the guinea-pig, on the contrary, is particularly resistant to anthrax infection provided we take care to protect the skin from infection. We know too now that its immunization is very easy, provided we take pains to vaccinate the skin.

A new idea then is brought forward, in regard to the mechanism of infection and immunity.—It pertains to the idea of the *autonomy of organs*. Each time that we deal with an infectious or toxic agent, it should be determined not only whether the animal is sensitive, but also whether there is a particular organ sensitive to the virus. We should also determime, if on vaccinating this elective organ, whether we will not succeed in obtaining an immunity, where the ordinary methods have failed.

The results of our research on cutaneous infection, cutaneous vaccination, and cutaneous immunity clashed with the prevailing opinions held in regard to anthrax. Directly, a number of publications appeared, in order to verify our conclusions. Even though the greater number

of authors confirmed the facts as we announced them, some regarded it as prudent to reserve opinion in regard to their interpretation.

The first experiments published, were those of Balteano[14] of the Hygienic Institute at Strasbourg. This author wished to determine whether all the organs possessed the same susceptibility for the anthrax bacillus, and further, whether the guinea-pig could be vaccinated by cutaneous vaccination. These experiments were made on rabbits and guinea-pigs. In a certain number of animals the virus was injected into the skin, in others it was introduced subcutaneously, intravenously, intraperitoneally or into the pleural cavity. In all these inoculations, precautions were taken to avoid injury to the skin.

The following are a few of these experiments.

In a series of rabbits 0.5 cc. of a 24-hour culture was inoculated into the marginal vein of the ear. None of the animals died.

Another series of rabbits received capillary tubes containing 0.3 cc. of a heavy emulsion of an agar culture into the peritoneal cavity. Three days later, on being assured that the peritoneal incision was entirely healed, the tubes were broken. The animals remained alive.

The third series of rabbits were injected with 0.5 cc. of a 24-hour culture of virus into the pleural cavity. No reaction occurred.

The fourth series received capillary tubes containing 0.3 cc. of a heavy emulsion of virus into the subcutaneous tissue—when the skin lesion was healed, the tubes were broken. The rabbits survived.

In order to control the virulence of the organism employed, Balteano applied the culture to the freshly shaved

[14] C. R. Soc. Biol., Vol. LXXXVII, July 22, 1922, p 653 C. R Soc Biol., Vol. LXXXVII, July 22, 1922, p. 655.

skin of a rabbit, and to another injected 0.1 cc. of the culture into the skin. The two animals died and anthrax bacilli were found in the blood.

After employing rabbits, this worker carried out some experiments with guinea-pigs. He found the same susceptibility for the skin in these animals, and the same non-susceptibility for the other organs—such as the peritoneum, circulatory system, and subcutaneous tissue. This point having been established, the author turned to vaccination.

He recalled the many attempts made by bacteriologists to vaccinate laboratory animals against anthrax. Following Pasteur's experiments on sheep and cattle, many workers tried to verify the results on guinea-pigs. The failures of R. Koch, Gaffky, Loeffler and others are well known. Balteano recalled this and then described his results.

On applying the first vaccine to the freshly shaved skin, and later repeating the procedure with the second vaccine, the animals became so solidly vaccinated that they would resist a subsequent inoculation of non-attenuated virus: his animals resisted the inoculation of 2 cc. of a broth culture of virus, or still better 1 cc. of a heavy emulsion of an agar culture. Guinea-pigs that had been prepared by vaccinating the skin subsequently resisted an inoculation of virus, when injected by any route. Large doses of virus could be inoculated intraperitoneally, subcutaneously or intramuscularly without taking the least precaution. All the control guinea-pigs that had received 0.1 cc. of the same culture by subcutaneous injection died of an anthrax septicemia.

Is this dermal sensitivity in regard to the anthrax bacillus a general phenomenon? In other words, is this susceptibility of the skin present in other animals, in the same manner as it is in the guinea-pig and rabbit?

It is known that the ox is quite insensitive to experimental anthrax infection. It is difficult to produce a fatal disease in this animal after subcutaneous inoculation of culture or of anthrax infected blood.

Following our experiments on skin infection in the guinea-pig, Vallée[15] desired to determine whether an intradermal inoculation in the ox would not be more severe than a subcutaneous one. Starting with this idea, he inoculated five calves intradermally with an organism obtained from the bone marow of an ox that had died of an anthrax infection. Each calf received 0.1 cc. of a broth culture intracutaneously.

The results were quite as Vallée had foreseen, for the intracutaneous inoculation of the virus produced disastrous results. Of the five calves, two died of anthrax in 65 and 90 hours respectively. The three others were very ill. The temperature rose above 41°C., and at the point of inoculation a lesion developed, which closely resembled the malignant pustule in man.

The ox then has a skin sensitivity quite like that found in the guinea-pig and rabbit. The same results were observed in sheep by Mazucchi.[16]

The rôle of the skin in anthrax infection has received further corroboration from the interesting experiments of Boquet.[17]

This author fed anthrax spores to fasting guinea-pigs. He found that usually the animals remained well after this procedure—only one of six guinea-pigs dying of anthrax. It is known, since the experiments of Pasteur, Roux and Chamberland, that laboratory animals, particularly the

[15] Bull. Soc. Centr Méd. Vét., July 30, 1923, pp. 285-288

[16] Clinica veter., July 1, August 31, 1923.

[17] C. R Acad. Sciences, Vol. CLXXVIII, January 7, 1924, p. 260.

guinea-pig, will resist infection following ingestion of bacteria, as long as the intestinal mucosa remains intact. It appears that the latter tissue reacts quite like the skin. We may state then that the entire covering of the animal—whether it be cutaneous or mucous—is refractory to the anthrax bacillus, as long as these tissues remain intact.

As was found in those guinea-pigs that had survived injection of virus into the peritoneal cavity or into the trachea—those that had ingested virus did not develop the slightest degree of immunity. For, these animals when tested with a small dose, for example, 0.1 cc. or 0.01 cc. of the second vaccine by subcutaneous injection, died in the same period of time as the controls.

Boquet made an interesting observation during the course of his experiments. He found a positive blood culture in 6 of 10 animals that had ingested the virus.

Of no less importance was the observation, that when guinea-pigs that had ingested virus were bled for culture by cardiac puncture, from 2 to 20 hours after the feeding, they all died of anthrax infection. At autopsy the characteristic edema was found at the point where the needle penetrated the skin. What caused the death of these animals? Boquet reasoned that doubtlessly, it was the traumatism caused to the skin by the puncture. For guinea-pigs of the same series, that had ingested the same dose of virus, but which were not submitted to this traumatism survived, in the proportion of 5 out of 6. In another series of experiments, of nine guinea-pigs that had ingested bacteria, only one died of an anthrax infection. On the other hand, all the guinea-pigs of the same series that had ingested bacteria died of anthrax, following shaving, irritation or contusion of the skin.

It follows from these experiments, that in order to produce a fatal anthrax infection, it is necessary to injure

the skin. "The hypothesis of Pasteur pertaining to the origin of intestinal anthrax," concludes Boquet, "and the new idea formulated by Besredka in regard to the extreme sensitivity of injured skin to bacterial infection, are confirmed by these facts."

An observation quite like that of Boquet was made by us at the outset of our studies on anthrax. Having determined that there were no ill-effects following an intraperitoneal inoculation, we wished to determine what happened to the bacteria that were introduced into the peritoneal cavity. We proceeded to withdraw some of the exudate at varying intervals, with a thin pipette. We found that following this procedure, all of our guinea-pigs died. Other guinea-pigs that were inoculated in the same manner, but which were not subjected to the peritoneal puncture, survived.

Following this observation we noticed that it was only necessary to introduce a needle into the peritoneal cavity which contained the bacteria, withdraw it immediately, and to find that the guinea-pigs died of anthrax, directly thereafter. We sought the explanation of this for sometime. It is now known. The bacteria which are inoffensive as long as they remain enclosed in the peritoneal cavity, become dangerous, directly upon coming in contact with the skin.

It is obvious that the death of the animal is not caused by the few bacteria which are caught up by the needle in leaving the peritoneal cavity; the number of bacteria which are retained in the skin are too insignificant to produce a fatal skin infection. Death is due to the influx of new bacteria, and to the bacterial toxin, which are to be found in the subcutaneous pocket caused by the needle and which leads to the skin lesion.

The importance of the skin in anthrax infection was also definitely shown by the experiments carried on by Plotz.[18] If the skin is responsible for the infection, he questioned, then the virus introduced in the subcutaneous tissue, should remain inoffensive. The problem was reduced to the following: to have the virus penetrate into the subcutaneous tissue without touching the skin.

The problem, which appeared difficult, was not insoluble. An artifice that Balteano had already employed permitted Plotz to overcome the difficulty. Instead of inoculating the virus with a syringe as is ordinarily done, he inoculated it "by capsule." He placed from 1 to 3 cc. of the virus in capsules of gelatine, collodion and glass, and so introduced the bacteria into the subcutaneous tissue. He then waited 3 or 4 days, until the skin wound was completely cicatrized. When he thought this was brought about, he liberated the anthrax bacilli by breaking the capsules.

These experiments showed that the virus could be liberated freely in the subcutaneous tissue; it in no way harmed the animal. In the experiments made by Plotz, the rabbits resisted from 1 to 3 cc. of the virus, even though 0.001 cc. of the same culture killed the control animals after intracutaneous injection.

Was not this insensitivity of the rabbit to the bacterium due to the fact that the culture lost its virulence while remaining under the skin? Plotz carefully controlled this, for he found that the virus, which had remained in capsules under the skin, even for 6 days, did not undergo any attenuation. It is seen then that virulent bacteria become inoffensive, when they came in contact with the subcutaneous tissue, and isolated from the skin.[19]

[18] Annales de l'Institut Pasteur, February, 1924, Vol. 38, p. 169.

[19] Analogous results were obtained by Hababou in rats C. R. Soc. Biol., Vol. XC, March 29, 1924, p 849

It should be noted that Plotz also met with failures, for rabbits that carried these capsules occasionally died of anthrax. But he was able to show that these deaths were due to the fact that the virus was liberated before the skin had entirely healed.

We believe that the negative results obtained by Bachman, Beltrami and Romat[20] were due to the insufficient cicatrization of the skin. In order to prevent the soiling of the skin with virus at the time of inoculation, these workers cauterized the point of inoculation in the ear with a hot iron. They state that the skin and even the auricular cartilage were destroyed. The greater the traumatism, the longer is the period of cicatrization. In these experiments, the bacteria then have ample time to return to the point of inoculation, and to infect the skin.

We are led to believe that to the same cause may be attributed the recent submucous injection experiments made by Boquet.[21] This worker succeeded in producing a fatal anthrax infection in guinea-pigs following the inoculation of the second vaccine under the buccal mucosa or under the conjunctiva. He concludes that the guinea-pig may contract anthrax other than by way of the injured skin.

One can not help but make a lesion in the mucosa at the time of the insertion of the virus, for we know from the experiments of Pasteur, Roux and Chamberland, that this is sufficient to produce a fatal anthrax infection. We feel that new experiments should be performed to show the actual susceptibility of the submucous tissue to the virus.

A number of investigators have observed fatal anthrax infections, without finding an edema at the point of inocu-

[20] C. R. Soc. Biol., 1923, 89, p. 1122.
[21] C. R. Soc. Biol. Vol. XC, January 19, 1924, p 72.

lation. They felt justified in concluding that the skin was not the only organ receptive to the virus.

It should be noted, that in all instances where no edema was found, the animals were inoculated with so-called "very virulent" bacteria; with anthrax infected blood, with encapsulated bacteria, or with bacteria to which so-called aggressin was added. We believe that these deaths, without an initial edema, which we have also observed, are due less to the bacteria themselves than to their products of secretion.

It may be objected, that we have not recognized the anthrax toxin *in vitro*. But this does not preclude the possibility of its existing *in vivo*. What is the significance of the great scarcity of bacteria in the edematous exudate—a fact known for some time and recently confirmed by histological studies of the skin?[22] How may we explain the possibility of vaccinating against anthrax with the edematous liquid, were it not for the presence of a soluble derivative in it? Has not the possibility of an anthrax toxin been indicated as a result of the experiments of Matsumoto,[23] who obtained the phenomenon of flocculation (Ramon test) by adding the edematous liquid of the rabbit, either to the blood of rabbits dead of anthrax or to the serum of rabbits immunized with edematous liquid freed of bacteria?

Always when we inoculate an animal with "very virulent" bacteria, encapsulated bacteria, "animalized" cultures, microorganisms and edematous fluid or, simply with anthrax infected blood, we inject in reality, more of "toxin"

[22] "In no instance are bacteria found free in the epidermis or in the derma of an animal dead of an anthrax septicemia. When the surface of the skin is inoculated, microscopic examination reveals no bacteria, even when the skin is excised at the height of the local reaction" (Rivalier, Thesis, Paris, 1924).

[23] Zeitschr F. Immunitätsf I Origin., t. July 30, 1925, p. 411.

than of bacteria. When we think that dysentery bacilli injected under the skin, are subsequently found in the intestine, as we will show later, we should not be surprised to find that the soluble derivative of the anthrax bacillus, which we will name toxin for the present acts directly on the cutaneous tissue. Two alternatives may occur: either the toxin penetrates into the skin in minute quantities, saturates the receptive cells and so creates a cutaneous immunity, or the toxin appears in a massive amount, and paralyzes the activity of the receptive cells of the skin; that is to say, interferes with their phagocytic activity. In this instance the bacteria which closely follow the toxin, find themselves in the presence of non-resistant cells; the local edema, which is the expression of the defense of the cutaneous tissue, is then not produced.

This is, according to our idea, the mechanism of anthrax infection without edema. This conception fits in with what we know of the function of phagocytes in general, and with the idea that we hold regarding the skin as being the susceptible organ in anthrax.

The experiments of Pasteur and his co-workers have demonstrated that the intestinal mucosa is not impermeable to the anthrax bacillus. The mucosa may be penetrated when it is injured by a sharp object. The intact mucosa constitutes, in the majority of instances, a barrier which the bacteria can not pass.

We now know of a certain number of viruses that may easily pass the intact mucosa. Since the experiments of Calmette, Guerin and Grysez, the possibility of the entrance of the tubercle bacillus by way of the conjunctival sac is known. We also know of the disastrous results that may follow the instillation of the plague bacillus in the eye. The virus of rabies, of vaccinia and of glanders

may also pass the ocular conjunctiva without the least difficulty. None of these viruses, it should be noted, produce a local lesion—they all attack the intact cells. They pass it, and then give rise to a generalized infection, which is usually fatal.

Considering the great virulence of the anthrax bacillus, we may expect a similar phenomenon to take place after instillation of the virus in the conjunctiva. Since the workers were not in accord regarding the effect of such an inoculation, our co-worker Aïtoff[24] studied this subject.

The experiments were carried on with mice, rats, guinea-pigs and rabbits. To obtain a heavy emulsion of bacteria, young agar cultures were washed off with a small quantity of salt solution. One or more drops of this emulsion were dropped directly into the conjunctival sac, without touching the membrane, and taking care to cause no injury.

None of the animals inoculated in this manner died. None presented the slightest reaction. In all instances however, bacteria could be found in the conjunctival sac the following day, and in a few instances bacteria could be found after seven days. In the majority of cases they disappeared after the second or third day.

Since the bacteria in anthrax infected blood are more virulent than cultures, the blood from a guinea-pig dead of anthrax was instilled in the eyes of guinea-pigs and mice. This blood, which contained numerous bacteria, also proved inoffensive. Of six mice inoculated in both eyes, none presented either a local or general reaction. Of eight guinea-pigs, submitted to the same treatment, two died of anthrax. Both of these animals happened to be pregnant. The six others remained well.

It follows from these experiments, that the conjunctival membrane constitutes an impassable barrier to the anthrax

[24] Ann. de l'Institut Pasteur, Vol XXXVI, p. 567, July, 1922.

virus; that the virus deposited there may remain alive for about seven days; that it may produce no disturbance, and finally is destroyed without causing any harm.

During the course of our experiments on infection and vaccination by the tracheal route,[25] we made a number of observations that may be of interest here. We found that soluble poisons, such as diphtheria toxin or cobra venom, are particularly active when injected into the trachea. The fatal dose by the respiratory route is almost equal to that following intravenous inoculation. These toxic substances easily pass the alveolar layer—one might even believe that they are sucked up by the lungs.

The conditions are different when we turn from soluble to insoluble elements, such as bacteria. The epithelial layer first intercepts the bacteria, and being held up in this manner they are slowly dissolved and finally pass the pulmonary filter. Because of this slow solution, which varies with each bacterial species, the tracheal route appears to give less severe reactions than the circulatory route. We had occasion to observe this sometime ago, with paratyphoid B and diphtheria bacilli. We found that inhaled viruses find a barrier in the alveolar filter, quite like the barrier of the intestinal mucosa, to certain ingested viruses.

The pulmonary filter is however far from completely stopping the bacteria. The tracheal route, even when intact, is not impenetrable. The majority of pathogenic bacteria which penetrate it induce the usual pathogenic reaction. Certainly, the intratracheal inoculation is less severe than an intravenous injection; but it is usually more severe than a subcutaneous inoculation and especially much more severe than an intracutaneous injection. In order to demonstrate this, we have only to recall the lethal

[25] Annales de l'Institut Pasteur, Vol. XXXIV, p. 362, 1920.

dose for the viruses of cholera, typhoid and dysentery bacilli, after injection by each of these routes.

The anthrax bacillus acts quite differently from the other viruses. Its inocuousness surprised us at the beginning of our studies, for we could inject enormous amounts of bacteria into the trachea, without producing any harm to the animal. This unexpected insensitivity seemed particularly striking when compared with the great susceptibility of the skin. The vulnerability of the organs for anthrax infection is completely reversed when compared with that for most known pathogenic bacteria.

We may conclude then, that the intact intestinal mucosa is a barrier to the entrance of the anthrax bacillus. It is not the only one, for the conjunctiva, as well as the lining of the respiratory system act in the same manner.

The problem of anthrax immunity is intimately associated with the susceptibility for the anthrax bacillus—for only skin infection permits us to obtain skin immunity. Since an anthrax infection can only take place in the skin, we can not conceive of an anthrax immunity unless it is the result of cutaneous immunization.

We have already called attention to Balteano's experiments concerning cutaneous vaccination in guinea-pigs. Let us also review those made by Plotz on cutaneous vaccination in rabbits.

This worker, after injecting rabbits with vaccine by the intracutaneous route, succeeded in obtaining a resistance in them as much as five hundred times the lethal dose of virus (lethal dose 0.001 cc.). On the other hand, rabbits prepared by subcutaneous inoculation with very large doses of culture (1 to 3 cc. in capsules) showed only a slight immunity or none at all.

Brocq-Rousseu and Urbain[26] had the opportunity of verifying the same results in guinea-pigs, which had received very large doses of anthrax virus intratracheally. These guinea-pigs, when subsequently inoculated intracutaneously or subcutaneously did not resist even a minimal lethal dose of virus.

Active anthrax immunity cannot therefore be obtained following either subcutaneous or intratracheal inoculation. Only vaccination of the skin confers a solid immunity. In other words, the results with the anthrax bacillus follow the principle, that immunization closely follows infection. The same cells, which we call receptive, react in both processes. We will later cite other examples which will bring out this relationship between immunization and infection.[27]

[26] C. R Soc Biol., Vol. XC, January 18, 1924, p 4

[27] The English bacteriologist Ledingham, has expressed the opinion that the bacteria do not kill the guinea-pig after intraperitoneal inoculation, because "the local peritoneal defences are sufficient to ward off a general infection" He adds that "the skin appears to be particularly vulnerable to experimental inoculation, simply because the local defense is of a low order." This explanation would have the advantage of conforming with the current opinion regarding the rôle of the leucocytes in immunity In order to accept this explanation, it is necessary that all viruses act as does the anthrax bacillus, but we know in reality, that it behaves in an altogether different manner.

Whether we deal with the Eberth bacillus or other bacteria of this group, whether it be the cholera vibrion or the pathogenic cocci, an intraperitoneal inoculation always proves fatal, in spite of the local peritoneal defence. The same viruses, on the other hand, inoculated subcutaneously or into the skin are almost always inoffensive, in spite of the feeble defence of the skin. If the local defences are as Ledingham believes, why does it only occur in anthrax and not in other infections?

Ledingham's discussion of the paper by Besredka, read and discussed by Plotz, before the Royal Society of Tropical Medicine and Hygiene. Transactions of the Royal Society of Tropical Medicine and Hygiene, 1924. Jan , Vol XVII, Nos 6 and 7, p 346

Following the experiments on guinea-pigs and rabbits, cutaneous vaccination was attempted on larger animals, such as sheep and horses.

The first experiments on the horse were made by Brocq-Rousseu and Urbain.[28] Because of the great sensitivity of the horse for the anthrax virus, these workers proceeded very cautiously. The first horse received two injections of the first vaccine and later three injections of the second vaccine *into* the skin. Then they inoculated gradually increasing doses of virus—the last being 5 cc. of a broth culture. During the entire period of the experiment, the temperature of the animal remained about normal. The search for antibodies—such as agglutinins, precipitins and complement-fixation—resulted in failures. The serum from this actively immunized horse could not protect the guinea-pig against an anthrax infection.

The second horse was also vaccinated by the cutaneous method, but in two stages. To the third horse, Brocq-Rousseu and Urbain decided to give the two injections at an interval of three days. The temperature of the three horses remained normal during the entire experiment. The search for antibodies in all resulted in failure.

These results showed that even in large animals, cutaneous immunization was possible—that this immunity was solid and could be obtained rapidly. Studies on the serum of these horses showed, that their immunity was far from running parallel with the quantity of antibodies present—for most often the antibodies were absent, or practically so.[29]

[28] Bull Soc Centr Méd. Vétérinaire, Vol. XCIX, No. 24, December 30, 1924, p. 482

[29] Gratia, one of Bordet's pupils, declared (C R Soc Biol , Vol. XCI, 1924, p 795) that he had found agglutinins and protective properties in the serum of guinea-pigs which had been cutaneously vaccinated This appears contrary to our results and those of Urbain and Brocq-

Following the first experiments on the horse an extensive study was made in the Army of the East last year, by the Chief Veterinarian Nicolas.

The animals in this army were fed with hay and barley obtained in the vicinity. Since the arrival of the French troops in Syria, anthrax had claimed many victims, for the troops patrolling in the wheat fields were often infected. Vaccination by the Pasteur method, carried on in an infected area in 1919, had to be abandoned because it proved too dangerous. Sero-vaccination carried on in 1920, 1921 and 1922 was only partly effective, and was also abandoned. Serumization, attempted in 1923 likewise did not give the hoped-for results. An epidemic of anthrax which occurred at Alep in August 1923, caused 25 deaths—the serum did not reduce the mortality, when employed before the eleventh day.

In view of these results, the chief veterinarian decided to employ cutaneous vaccination on all the horses of the army, which numbered about 10,000 animals.

Rousseu. Gratia believed that the serum of guinea-pigs which had been cutaneously vaccinated, had a protective property, because 0 1 cc. of virus injected *in mixture* with 2.5 cc. of this serum did not kill the animal. This neutralization of the virulence of the anthrax bacilli is not surprising to those who know the great sensitivity of the anthrax bacillus for all foreign liquids, and in particular for an agglutinating serum. Sometime ago we found an analogous reaction with the typhoid bacillus to normal beef serum, which is more resistant than the anthrax bacillus. After having submitted the typhoid bacilli to the agglutinating action of beef serum, we were able to inject guinea-pigs with many times the fatal dose, by intraperitoneal injection We never thought of considering the natural immunity of the ox, or the protective properties of its serum. Normal beef serum, which is active in mixture, is devoid of all action when it is injected apart from the virus. The same holds true for the serum of guinea-pigs which have been cutaneously vaccinated Gratia has shown that this serum shows no protective properties against the virus, when the serum and virus are introduced separately, in two different parts of the animal body.

A preliminary experiment was made on 72 horses, in order to prove the harmlessness of the method. The outcome was favorable. The injections of the anthrax vaccines *into* the skin produced no general reaction, and the animals were able to return to work the following day. In six instances a local reaction followed, which consisted of an edema at the point of inoculation.

Since the reaction following vaccination was slight, universal vaccination by the cutaneous method was ordered in the Army of the East on January 15, 1924. This was done by employing two injections at six-day intervals with the two Pasteur vaccines. The first vaccine was injected into the skin of the shoulder, employing 0.25 cc. This amount of vaccine was divided in three injections, (extensive cutaneous vaccination)[30] each injection being on a vertical line, 5 cm. apart. The second vaccine was injected on the seventh day, employing 0.25 cc. and the injections were made as before, on the other shoulder. All of the results of this antianthrax campaign to January 1, 1925, or one year after the beginning of the vaccinations, are summarized here, and are published in a report by the veterinarian, Major Lebasque.[31]

Of 8,912 horses and mules, cutaneously vaccinated in 1924, there were 4 deaths. Two occurred during the course of the vaccination, and two later. This is an incidence of 0.45 per thousand. During a period of 5 years—from 1919 to 1923—there were in round numbers, 88 deaths per year or 8.1 per thousand.

Cutaneous vaccination had then reduced the average mortality in the 5 past years about 20 times. "This is," concludes the Director of the Veterinary Service Nicolas, in his report to the Minister of War, "a success without

[30] Cutivaccination en nappe

[31] Revue Veterinaire Militaire, Vol. VIII, p 197.

precedent in the history of antianthrax vaccination in horses; a success due, to the complete harmlessness and efficacy of cutaneous vaccination."[32]

The inocuous nature of the reaction after cutaneous vaccination is quite in contrast with the severe reaction in horses which usually follows vaccination by the subcutaneous route. Only recently, during an epidemic of anthrax in the department of the Ain, the Veterinarian Saint-Cyr recorded very serious post vaccinal accidents. All precautions were taken to prevent reactions in these sensitive animals. Four or five days after subcutaneous injection of the second vaccine, 7 horses developed serious symptoms, and three died of anthrax. Following these vaccinations this worker states, "that after the results obtained, we hesitate greatly to vaccinate horses in the future."[33]

The animal that benefits mainly from antianthrax vaccination in France is the sheep. Mazucchi[34] made the first experiments with this animal. This worker found that he could succeed in conferring a solid antianthrax immunity on sheep after intracutaneous inoculation with increasingly virulent vaccines. These animals subsequently resisted inoculation of virus by any route, without showing any other reaction than a moderate elevation of temperature.

Another experiment of the same order was made in

[32] Nicolas, Antianthrax cutaneous vaccination in horses and mules in the Army of the East in 1924. Revue Véterinaire militaire, Vol. IX, p. 54, March 31, 1925.

[33] Saint-Cyr, Bull de la Soc. des Sciences Veter. de Lyon, November-December, 1923, p 327.

[34] Clinica Veter , July 1–August 31, 1923.

Morocco by Velu.[35] Eleven sheep were cutaneously vaccinated, the inoculations being given at one time. All the animals did not however receive the same dose. The doses varied from $\frac{1}{6}$ to 20 doses, taking as unity the ordinary amounts employed for subcutaneous injection. The amount of vaccine inoculated by the cutaneous method was equivalent to 20 doses—to 2 doses—to 1 dose—to $\frac{1}{2}$ dose—and to $\frac{1}{6}$ of a dose.

Following the vaccination many of the sheep developed a slight transitory edema and slight rise of temperature; while others presented no reaction at all. All the sheep, cutaneously vaccinated, were submitted to an inoculation of virus 14 days later. The virus employed was a very active Moroccan strain, which was inoculated subcutaneously. The dose employed, represented 5,000 times the lethal dose for the rabbit.

These are the results obtained: The sheep that received $\frac{1}{6}$ of a normal vaccinating dose into the skin died of anthrax. The animal that was injected with $\frac{1}{2}$ of a vaccinating dose into the skin developed an edema and a sharp rise of temperature—but survived. All the other sheep cutaneously vaccinated, survived without exhibiting the least reaction.

The experiment was convincing. It indicated that a solid immunity may be obtained in the sheep after employing a single injection of the vaccine, inoculated into the skin.

Laying stress on the results of these laboratory experiments, Velu felt justified in applying them to practice. During the year 1924, he succeeded in vaccinating in Morocco, with the co-operation of the Chief Veterinarian, Th. Monod, 21,640 cattle (14,405 oxen; 12,520 sheep; 4,640 pigs; and 75 horses). These vaccinations were

[35] C. R. Soc. Biol., Vol. XC, March 28, 1924, p. 746.

carried on in a heavily infected area, either on herds living in infected pastures, or during an epidemic of anthrax. The injections were all made *at one time into the skin.*

Most of the practitioners who performed this experiment were in accord, in affirming that the intradermic inoculation was simple and easily applied. Velu alone was able to make 600 vaccinations in one day; that is to say, 1,200 injections in the half savage cattle. These intradermic injections were followed by no reaction, while those made under the skin with the same vaccine, produced various symptoms: local, thermal and general.

As a result of these observations the authors concluded, that the intradermic vaccination is at least as efficacious as subcutaneous vaccination; that it is inoffensive in contaminated herds; the rapid appearance of the refractory state permits the vaccination to be made in an infected area, without previous serumization. Because of its simplicity, its efficacy, its moderate price, intradermic vaccination becomes the method of choice in countries of extensive cattle raising.[36]

Anthrax was the first disease in which the rôle of bacteria was definitely shown. It was the first important infectious disease that was benefited by vaccination with an attenuated virus. It was while studying anthrax that the doctrine of cellular immunity took form.

Did we not think, when Behring in 1888 found that the rat, a refractory animal to anthrax, possessed a bactericidal serum, that we had the key to the question of anthrax immunity and perhaps to immunity in general? We needed the well-known experiments of Metchnikoff, published in 1890, in order to point out the real importance of

[36] Th Monod and H. Velu. The intradermo-vaccination at one time against anthrax, and its advantages. C R Soc. Biolog., Vol. XCII, January 31, 1925. See also the Rapport of the Director of Agriculture at Rabat.

this immunity in the rat. From this time on the problem of antianthrax immunity seemed to enter the general scheme of the phenomenon of immunity. No one believed that anthrax held any more surprises for us. Did not the animals dead of anthrax, show the classical picture of a septicemia? Do not the bacteria circulate in the blood and in the organs?

We met with general opposition when we declared that this deadly virus attacked in reality, only one group of cells—namely the skin and the mucosa.

Today, the story of anthrax, looked at from the point of view of the mechanism of infection and immunity may be summarized in the following two propositions:

1. The susceptibility of the animal is limited principally, if not exclusively, to the cells of the skin.

2. The immunity of the animal is due to the vaccination of the receptive cells.

It should be pointed out that cutaneous vaccination is not followed by any production of antibodies. Anthrax is hence the type disease illustrating local immunization. If the larger animals—sheep, cows, horses—may be vaccinated by the subcutaneous route, we feel that their immunity is due to the cutaneous traumatism caused by the inoculating needle. The needle, by producing a sort of tunnel or subcutaneous pocket permits the bacteria to return to the skin, to infect the receptive cells (cuti-infection), to vaccinate them (cuti-vaccination) and so creates an antianthrax immunity (cuti-immunity).[37]

[37] The term cuti-vaccination has been employed by certain authors to indicate a different meaning from what we give it. For instance, it has been wrongly used to indicate that an antigen (red blood cells, for example) may be injected through the skin, in order to vaccinate On creating this new word, we not only had in mind the idea of indicating the route employed for vaccination, but particularly to call attention to the part played by the skin and the "cuti-immunity" (cutaneous immunity) that follows.

CHAPTER II

Staphylococcus and Streptococcus Infections

Relationship between the staphylococcus and streptococcus, and the anthrax bacillus. Difficulties of active and passive vaccination. Affinity of the staphylococcus and streptococcus for the skin.

I. *Antistaphylococcus vaccination.* Lesions produced in normal guinea-pigs after subcutaneous injection of the staphylococcus. Lesions produced in vaccinated guinea-pigs after injection of cultures, under the skin, into the skin, or after application on the skin. Vaccination by means of wet dressings. The rapid appearance of immunity excludes the possibility of the participation of antibodies and argues in favor of a local immunity.

Filtered staphylococcus cultures or "antivirus." Its characteristics; inhibitory action on the staphylococcus, resistance to heat; specificity; affinity for cutaneous tissue; vaccinating power, feeble vaccinating action by other means than by way of the skin. Wet dressings with staphylococcus antivirus. Experiments on rabbits

Antistreptococcus vaccination. Difficulties of vaccinating man and laboratory animals. Cutaneous vaccination with killed cultures; extensive cutaneous vaccination. Wet dressings of antivirus in guinea-pigs Corresponding results with those obtained with the antistaphylococcus wet dressings. Comparative results of vaccination in guinea-pigs by injection under the skin and into the skin. Local immunization in rabbits against the streptococcus by injection under the skin and into the pleural cavity. Ocular infection in the guinea-pig and rabbit. Local immunization against the streptococcus, staphylococcus and pneumococcus. Histologic study of the vaccinated and non-vaccinated cornea.

II. *Function of the skin and mucous tissues in human staphylococcus and streptococcus infections* Localization of the staphylococcus in man: external ear, middle ear, conjunctiva, vagina, uterus, nasal cavity, etc. Localization in bones and joints, cardio-vascular and renal systems; early stages of infection in the skin. Routes of infection.

Vaccinotherapy. Observations. Furuncle of the ear; varicose ulcer, furunculosis, psychosis; folliculitis; boil on the chin; osteomyelitis; pyodermatitis in the new-born, pleural fistula; puerperal infection; chronic conjunctivitis, phlegmon of the lacrymal sac; injury of the cornea; ulcero-membranous blepharitis; bilateral dacryocystitis. Method of action of filtered cultures in man. Antiseptic and aseptic dressings. Advantages of specific dressings.

In the realm of pathogenic bacteria individualism is developed to a very high degree. All intermediary stages may be found between bacteria that are very infectious and those that act entirely by virtue of their toxin. It is difficult to find two strains of bacteria that resemble each other, in regard to their manner of attacking the organism. The manner in which bacteria develop immunity, varies also with different strains. While in anthrax, a local immunity is the principal factor, in other infections, a general immunity may take part in the defense of the organism. But irrespective of the part that a general immunity may play, there are groups of diseases, which may be compared, if they are regarded from the standpoint of the mechanism of infection and of immunity.

There are few bacteria that appear less comparable than the anthrax bacillus on the one hand, and the staphylococcus and streptococcus on the other. Their biologic properties are as widely different as their morphology. They have however, one characteristic in common, and that is a negative one; namely, their inability to serve as antigens. It is difficult to vaccinate against the staphylococcus or the streptococcus. In this respect they resemble the anthrax bacillus, that is before we knew of cutaneous vaccination. The failure to immunize with the anthrax bacillus was explained on the ground of its great virulence; but as far as the cocci

under consideration were concerned, the failure could not be explained in the same way, for they were generally innocuous for laboratory animals. It is nevertheless almost as difficult to vaccinate the guinea-pig against the staphylococcus, and in particular the streptococcus today, as it had been to vaccinate this animal against the anthrax bacillus previously.

We can not enumerate the many attempts that were made to prepare antistaphylococcus and antistreptococcus sera. The attempts to demonstrate the presence of antibodies—bactericidal and others—in the blood of man and animals, after infection or cure from disease, were also numerous. While it is impossible to review these researches, because they are as varied as they are contradictory, there is a conclusion which may be drawn; namely, that it is difficult to vaccinate with the staphylococcus and the streptococcus, and further that these bacteria do not develop antibodies. The current methods of immunization, consisting in injections under the skin, into the peritoneum or into the vein, are hardly efficacious—especially insofar as the streptococcus is concerned. This failure is explained, by stating that the guinea-pig does not develop antibodies. But we should note, that man does not develop antibodies either; yet in certain instances he may derive some benefit from staphylococcus vaccination. The reason for the failures therefore, does not depend upon the presence of antibodies.

The favorable results of vaccino-therapy in man proves that antistaphylococcus immunity does exist. Let us then have the courage to admit, that in spite of all the explanations which have been given, we do not understand the mechanism of this immunity.

Enlightened by the experience with anthrax, we con-

sidered whether the difficulties encountered in animals with staphylococcus and streptococcus vaccination, were not due to the same factors which prevail in the case of the anthrax bacillus. Is it not here too a question of the route of penetration of the virus and the receptive cells?

While the staphylococcus does not possess an absolute affinity for the skin, as does the anthrax bacillus, still it shows a predilection for the cutaneous tissue. If the favorable results of vaccino-therapy in man are due to a local immunization, as indicated by our hypothesis, would it not be more rational to aim at the skin, and leave aside the question of the production of antibodies, as the doctrine of Wright indicates? Instead of making the injection subcutaneously or intravenously, as is generally done, would it not be better to vaccinate the receptive organ? On following this reasoning, we thought that man, who is more sensitive than animals to streptococcus and staphylococcus infection, may also be more easily vaccinated by this method. The small dose of vaccine employed to vaccinate man many not be sufficient to vaccinate the animal. If this supposition were correct, could we not obtain an immunity in laboratory animals by vaccinating the cutaneous apparatus?

The majority of staphylococci or streptococci isolated from man, are only slightly pathogenic for animals; they therefore can not be employed for vaccination experiments. The strains employed in our studies were very virulent for the guinea-pig; and so we were able to undertake a series of studies with reference to the elucidation of the mechanism of infection and immunity.

I

The staphylococcus employed in these experiments produced a characteristic skin lesion in the guinea-pig, following a subcutaneous injection in the abdominal region, of 1 cc. of a 24-hour broth culture. After a latent period of 24 to 48 hours, an area of edema developed at the point of inoculation, which spread from day to day, so that it subsequently invaded a large area of the abdomen. The edematous tissue became hard and finally produced a scar. The skin of the infected area was red to begin with and rapidly became black and moist. A large scar was formed, which on being detached, revealed a bleeding area, which took a long time to heal. When a large dose of staphylococci was injected under the skin (0.5 to 2 cc.) the guinea-pig died in from 3 to 6 days of a septicemia.[1]

The picture is entirely different in prepared guinea-pigs. This preparation is made a day or two before by injecting killed staphylococcus cultures (60° for one hour) or filtered broth cultures.

Either one of these cultures are injected under the skin or into the thickness of the skin, depending upon the nature of the experiments. In other instances the cultures, filtered or non-filtered, are applied to the skin of the abdomen, in the form of a wet dressing, after previous removal of the hair and shaving. The test is made by inoculating a living staphylococcus culture under the skin,—24 to 48 hours or even 72 hours after this preparation.

On comparing the lesions produced in guinea-pigs prepared by the various methods, we note the following: In animals prepared by injecting into the skin, the lesions

[1] C. R. Soc. Biol., Vol. LXXXVIII, May 19, 1923, p. 1273

are more discrete than in those which received the vaccine under the skin. This difference is particularly marked in guinea-pigs which have been vaccinated by the application of wet dressings to the shaved skin. In these animals, instead of finding a deep scar, we only find a superficial lesion, or more frequently a circumscribed abscess, which soon opens and heals. The immunity which has developed in these prepared animals, appears to be clear.

When we consider that this immunity follows the simple application of a wet dressing, that it appears as soon as 24 hours after the application, it is apparent that the participation of antibodies may be excluded. Furthermore, the search for the presence of antibodies in the serum of these vaccinated animals always give negative results.

There appears to be an analogy between antistaphylococcus and anthrax immunity. In both instances cutaneous vaccination confers the greatest degree of immunity; in both cases the immunity is particularly localized in the skin; in both conditions the immunity is acquired independently of antibody production.

We have just seen that a dressing, soaked in a culture of staphylococcus may act as a vaccine. Considering that *corpora non agunt nisi soluta*, it was natural to suppose, that it was not the bacterial bodies themselves which acted, but rather their soluble products or the substances derived from the bacterial bodies. With this idea in mind we directed our experiments in that direction.

When we filter an 8- to 10-day broth culture of staphylococcus, we obtain a liquid, which at first glance appears quite like an ordinary broth. When this liquid is injected into an animal we find that it is free of all toxicity,

even though it was derived from a virulent culture. If this broth be reinoculated with various bacteria, a luxuriant growth takes place; quite like a culture in a normal broth.

Only the staphylococcus grows sparsely or not at all after reinoculation in this liquid. The bacteria retain their vitality, but fail to grow in it. The inhibitory action of the liquid may also be demonstrated, to a certain degree, *in vivo*. For when a guinea-pig is inoculated under the skin, simultaneously with 2 cc. of this liquid and 1 cc. of a 24-hour broth culture of staphylococcus, we find that the lesion produced at the point of inoculation is much less severe, than when the bacteria are injected alone, or with an ordinary broth. The filtered culture then contains a substance which paralyzes the activity of the staphylococcus. This inhibitory action is evident, either *in vivo* or *in vitro*.

What is the nature of this substance?

Our experiments have shown that it is a substance, or a property, which resists heat and which is specific. The filtered culture may be exposed at 100°C. for 30 minutes or even at 120°C. for 20 minutes, without interfering with its properties. The inhibitory action of this liquid reacts only on the staphylococcus. A filtered broth of another bacterium, such as the typhoid bacillus for example, is incapable of producing the same effect. We will show further on that this substance, taking for granted that it is one, has an elective affinity for the skin. quite like the staphylococcus from which it was derived. This substance diffuses easily in a liquid medium, as the method of its preparation indicates.

It appears then, that there are two substances within the staphylococcus. One, a thermolabile virus which is adherent to the body of the bacterium and which may

produce serious skin lesions or death of the animal. The other is atoxic, thermostable and is easily detached from the body of the germ and antagonizes the former substance. For convenience, we will call this substance, "antivirus." Both the virus and the antivirus derived from the staphylococcus are absolutely specific and both possess an elective affinity for the skin.[2]

Having shown the efficacy of vaccinating with wet dressings of heated staphylococcus cultures, the thought occurs that the vaccinating power of the dressings is probably due to the presence of antivirus in the cultures.

This idea was confirmed by experiments. As a matter of fact we found, that when we replaced the whole culture by a filtered one, the results were still better; for the vaccinating effect of the antivirus, becomes particularly marked depending upon whether it enters by way of the skin or by other routes.

Our experiments have shown that immunity may or may not develop, depending upon whether the vaccination was made cutaneously or intraperitoneally.

[2] C R. Soc Biol , Vol. LXXXIX, June 2, 1923, p. 7.

As a matter of interest, this is the idea which preceded these experiments. Like many others, we observed that certain bacteria rapidly lose their virulence, particularly in a liquid medium, when the culture is kept at 37° for more than 24 hours. This loss of virulence, which was observed in cultures of cholera, staphylococcus, streptococcus and other bacteria, attracted our attention. The explanation that is usually given, namely, the increase in age or death of the bacterium, did not appear convincing

Starting with the idea that in certain cells of the body, such as the white blood cells, we often find the ferment in association with its antiferment, we wondered whether if in the bacterial cells, there did not exist in association with the virus, an antivirus. We thought whether this antivirus, which is liberated by the aid of the temperature, 37°, does not pass to the exterior of the bacterium and so render it less virulent This was the reasoning that led to the separation of the antivirus

A protective dose of the staphylococcus filtrate, injected into the peritoneal cavity, is practically without effect. Is this due to the fact that the specific substance, injected in the dose employed, does not reach the receptive cells of the skin? We do not know. In this instance the antivirus is incapable of protecting the guinea-pig against death; the antivirus when injected in the peritoneum is even incapable of preventing the production of a skin lesion.

The filtered culture, injected under the skin, has a slight vaccinating effect, but its action is less than when it is injected directly into the skin. In the latter instance, the guinea-pig does not develop a skin lesion; the only lesion that may appear is a small superficial erosion of the skin.

The effect of intracutaneous vaccination is particularly clear-cut when many points of the skin are injected. We call this "extensive cutaneous vaccination."[3] Its purpose is to have a large area of skin partake in the vaccination process.

The greatest degree of immunity is obtained when the entire surface of the skin takes part in the process of vaccination. This may be accomplished by applying a dressing, soaked in the vaccinating liquid, to the entire abdominal surface. An animal treated in this manner will resist a fatal dose of staphylococcus, even though it was only prepared the preceding day. The area of skin inoculated in this instance shows nothing or only a slight localized superficial lesion in the epidermis. The control animal will develop a large warm indurated area which invades the derma and leads to an ulcer. The skin of the guinea-pig, which has received the application of the antivirus develops neither warmth nor redness.

[3] Cutivaccination en nappe

The only occurrence may be a collection of pus under the epidermis; the little abscess opens rapidly and the skin returns to its normal aspect.

At first glance it appears curious that the immunization of a limited area of skin should lead to an immunity of the entire surface of the skin, without this immunity being previously transferred by the blood, that is to say, without the immunity having been at a certain moment, general. We feel that such a supposition is illogical, and is contrary to fact. By practising cutaneous vaccination to a certain area of skin, we have in reality, an immunity limited to this area; it is rare that we immunize the entire animal.

The resistance is pronounced when the staphylococcus or streptococcus cutaneous vaccination is made over a large area, or still better, after applying the specific dressing to the entire abdominal surface of the guinea-pig. In this instance the character of the vaccination remains the same; that is local, but its effect reacts upon the rest of the cutaneous surface, because of the large net of lymphatic vessels interested in the process. The receptive organ par excellence, being in a large measure protected, the animal shows evidence of a solid immunity —and this takes place without the participation of antibodies. This is what happens when the immunity in the guinea-pig is established after the application of a wet dressing to the entire abdominal surface. The same is true after vaccinating with a living virus (vaccinia or others) which gradually reaches from the point of inoculation to the most distant areas.

To conclude: The vaccinating principle contained in the interior of the staphylococcus may be isolated and obtained in a dissolved state in a liquid medium. This principle is atoxic; thermostable and specific. It

only acts after inoculation into or after application on the skin. The slight vaccinating effect after subcutaneous or intraperitoneal inoculation, the appearance of immunity the day following its inoculation into the skin or after application to it, excludes the possibility of the participation of antibodies in the blood. This is in favor of the idea that it succeeds, because of action of the receptive cells of the skin or by a local cutaneous vaccination.

Let us briefly recall the experiments of Urbain,[4] who obtained identical results in rabbits, as those described by us in the guinea-pigs.

This worker wished to determine, to what extent rabbits could be protected against a fatal staphylococcus infection, after a previous application to the skin of a specific dressing. He found that in about half of the cases the animals were definitely protected and in the other half, life was prolonged by several days, beyond that of the controls. The test inoculation to which the rabbits were subjected was quite severe—for it consisted in an intravenous inoculation of 1 cc. of a 24-hour broth culture; a dose sufficient to kill the control animal in 6 days. The autopsy showed staphylococci in the blood as well as these bacteria in the multiple abscesses in the kidneys.

The immunity conferred to the rabbits by the antivirus, already appears 24 hours after the application of the wet dressing.[5]

[4] C. R. Soc. Biol., Vol. XCI, July 5, 1924.

[5] It appears that rabbits may be protected against a skin lesion if they ingest massive quantities of staphylococci, after a previous sensitization with bile (Combiesco and Calalb, C. R. Soc. Biol., Vol XCI, 1924, p. 734). If this be true, it may be explained by a sort of synergy which exists between the skin and the intestine. We are aware of

The streptococcus which is often in association with the staphylococcus, differs in many characteristics. They resemble each other in regard to their manner of vaccination. We know that the staphylococcus has little antigenic property. It is far from being a perfect vaccine, especially in laboratory animals; however in man we are more successful with antistaphylococcus vaccinotherapy.

When we attempt to vaccinate with the streptococcus we encounter insurmountable difficulties. Bacteriologists have tried many methods. Some investigators heated the streptococcus to 63°, while others put their faith in heating cultures at 60°; some added galactose and urea, others treated them with trichlorid of iodine; some employed young cultures, while others tried old ones. All procedures were in vain. For when the streptococcus is killed, whether it be by heat or chemical means, it does not vaccinate.

It should be noted however, that living streptococci may vaccinate; but in order to succeed, we must inject an amount that is almost equal to the fatal dose. This is a method of vaccination which, under stress, may be

intestinal disorders, often severe, which follow extensive burns of the skin. We know of the susceptibility to anthrax in large animals; that they may be infected by the buccal as well as by the cutaneous route. The same sensitiveness holds true for the virus of malta fever, glanders, tuberculosis, etc.

In referring to typhoid and paratyphoid infections, we expressed the opinion sometime ago that the receptive cells are probably in the intestinal glands. In the skin, the receptive cells are probably the reticulo-endothelial cells. In spite of the different embryologic origin, the mucous covering which protects the organs, may respond in the same manner as the cutaneous. The two routes of entry which the virus may take are often interchangeable. This is so definite that the immunization may follow with success by one or the other method. Practically, the route of elective vaccination is that which is the ordinary route of infection.

used in the laboratory, but has little chance of success in human practice.

Attempts at vaccinotherapy in man, by means of dead streptococcus cultures, are still being tried. Even today we employ this method, because we have nothing better. We are often encouraged by the good results obtained in staphylococcus infections. But it should be admitted, that with the streptococcus, the practical results are usually nil.

Following our experiments on antistaphylococcus cutaneous vaccination in the guinea-pig, Brocq-Rousseu, Forgeot and Urbain[6] attempted to employ the same method, for vaccination against the streptococcus. The results obtained by these workers were encouraging. While the injection of dead streptococci (60° for 15 minutes) under the skin, produced no immunity, yet when they were injected into the skin, 3 of 5 guinea-pigs were protected. When the intracutaneous injections were made over a large surface of skin, according to the principle of extensive cutaneous vaccination, rather than in one single place, they succeeded in protecting 7 of 8 guinea-pigs.

In other terms it is preferable to substitute extensive cutaneous vaccination for the ordinary method of vaccination. In this case we may even employ dead streptococci with success.

As soon as the antistaphylococcus dressings gave favorable results we began to study, in association with Urbain, the streptococcus antivirus and dressings prepared with it.[7]

We soon found that the streptococcus, when culti-

[6] C. R. Soc Biol., Vol LXXXIX, June 23, 1923, p. 219.
[7] C. R. Soc. Biol , Vol. LXXXIX, July 21, 1923, p 506.

vated in a liquid medium—serum broth or ordinary broth—permitted a specific substance to diffuse in it, which had all the characteristics of the staphylococcus antivirus.

The strain of bacteria employed in all our studies was very virulent for the guinea-pig. After a subcutaneous injection of 1 cc. of the culture, a characteristic skin lesion was produced; when 2 cc. of the culture was injected under the skin of a 400-gram guinea-pig, it died within 24 hours.

The wet dressings are prepared in the same manner as for the staphylococcus. The culture is grown in a liquid medium at 37° for 8 or 10 days, and is then filtered; the filtrate is again reinoculated and kept at incubator temperature for 8 to 10 days, and again filtered. The streptococcus will not grow again in this liquid.

We will cite a few experiments in which this filtrate was employed, in order to make our point clear.

1 Three guinea-pigs were prepared, by applying a wet dressing (to the shaved skin) of a streptococcus filtrate in serum broth.

Two guinea-pigs, serving as controls, were prepared by applying a wet dressing of serum broth alone. The dressings were removed after 24-hour application.

After a period of three days the test inoculation is made.

Three new guinea-pigs are added to the group of 5 animals that had already been prepared with the wet dressings. All the animals are inoculated under the skin, with 2.5 cc. of a young streptococcus culture in serum broth.

The following day the 3 control guinea-pigs and the 2 guinea-pigs which were prepared with wet dressings of serum broth alone, died. The only animals that remained alive were the three which were prepared with the wet dressing of the filtered culture.

2 In another experiment, instead of waiting 3 days after the application of the wet dressing, the test inoculation was made 24 hours after the application of the dressing.

Three guinea-pigs were prepared with antivirus wet dressings.

Two guinea-pigs were prepared with wet dressings of serum broth alone.

Two guinea-pigs acted as controls.

The following day the wet dressings were removed and all the guinea-pigs were inoculated with virulent streptococci, in the same manner as in the preceding experiment.

The only guinea-pigs that survived were those that were prepared with a wet dressing of the filtered culture.

3. Three guinea-pigs were inoculated subcutaneously with a mixture of 2 cc. of streptococcus antivirus and 2.5 cc. of an 18-hour culture of streptococcus.

Two control guinea-pigs were inoculated subcutaneously with a mixture of 2 cc. of serum broth and 2.5 cc. of the same streptococcus culture.

The next day the two control guinea-pigs were found dead of a streptococcus infection; of the three guinea-pigs that had received the mixture of the filtrate and the culture, one died after 60 hours, another after 4 days, and the third survived.

4. Two guinea-pigs were inoculated intraperitoneally with 0.5 cc. of an 18-hour streptococcus culture. This was the minimal lethal dose by intraperitoneal injection.

Another guinea-pig was inoculated intraperitoneally with a mixture of 0.5 cc. of streptococcus culture and 2 cc. of serum broth.

Two guinea-pigs were inoculated intraperitoneally with a mixture of 0.5 cc. of streptococcus culture and 2 cc. of streptococcus antivirus.

Three guinea-pigs, that had been prepared the day before with 2 cc. of the streptococcus antivirus, by intraperitoneal injection, were inoculated subcutaneously with 2.5 cc. of a streptococcus culture—at the same time as the other animals. None of these animals survived.

As a result of these experiments it appears that the specific substance developed in old cultures possesses an elective affinity for the cutaneous tissue. No protection follows an intraperitoneal injection, but the substance has a definite protective effect when injected under the skin. But the maximum effect of the antivirus follows its direct application to the skin, in the form of a wet dressing. In this instance the animal

is vaccinated against a cutaneous lesion or the lesion that follows the subcutaneous inoculation of the streptococcus. In place of the production of an ulcer, surrounded by a large inflammatory zone, we find only a small abscess in the midst of healthy tissue. Further, when a fatal dose of streptococcus is inoculated, the animal will be saved, provided that a wet dressing has been applied at least 24 hours before.

All of these facts, relative to antistreptococcus vaccination—whether it be by non-filtered cultures or antivirus—are comparable to the results described for the staphylococcus and its antivirus.

We must admit that a certain relationship exists between the cocci under discussion and the anthrax bacillus. This analogy will become closer we hope, when we will be able to isolate the anthrax antivirus.

The staphylococcus and the streptococcus to be sure, have not the same attraction for the skin as the anthrax bacillus. Still it is quite true, that even though these bacteria may localize in other places, they still have an elective affinity for the cutaneous tissue. Every time that the cocci are implanted in the skin, mucosa or other tissues, a strictly local infection is first induced. This lesion is the only evidence, for a certain period, of the infection. As long as the staphylococcus or streptococcus remains localized in this manner, it conforms to the methods of local immunization. We will return to this, but we should inquire whether vaccination of the organs or tissues—protective as well as curative—does not take place locally, without the assistance of antibodies.

Before going further, let us summarize the main facts that follow from our studies.

Killed cultures of *staphylococcus*, injected under the

skin, confers a certain degree of immunity against a cutaneous lesion; cultures injected into the skin, vaccinate much better than those injected under the skin.

Filtered staphylococcus cultures contain the same vaccinating principle as whole cultures, but the action is greater in the former. The substance contained in the filtrate, or antivirus, is most effective when it is brought into more intimate contact with the skin. The staphylococcus filtrate, applied to a limited area of skin in the form of a wet dressing, will protect this area against a cutaneous lesion—that is an ulcer; if the wet dressing is applied to a large surface of skin, almost the entire area of skin is protected; it protects the animal against a fatal dose of virus. The vaccinating effect of the filtrate is rapid and is not due to the production of known antibodies.

Killed *streptococcus* cultures, injected under the skin, confers no immunity to laboratory animals; when injected into the skin a certain degree of immunity is produced, and this immunity is augmented when the number of points of injection is increased (extensive cutaneous vaccination).

Filtered streptococcus cultures contain a specific antivirus. This antivirus possesses the same affinity as the culture itself. The antivirus is only active against virulent streptococci, when inoculated under the skin, and particularly after application to the skin. Wet dressings of the filtrate confer a solid immunity against a local infection in guinea-pigs and rabbits; when the wet dressings cover a large area of skin, the animal is protected against the inoculation of a fatal dose of virus.

The unexpected results of these experiments, on infection and immunity with staphylococcus and streptococcus

led other workers in different countries, to attempt to verify them. We will cite some of these experiments.

Frans de Potter isolated a staphylococcus from a case of osteomyelitis, which contrary to the rule, was virulent for the guinea-pig. When 0.5 cc. of an agar culture diluted in 10 cc. of salt solution was injected intravenously, it killed the animal; when 1 cc. was injected subcutaneously an abscess was produced which opened in 4 to 5 days.

This worker wished to determine the possibility of vaccinating guinea-pigs with this culture. A series of guinea-pigs were injected subcutaneously, with eight inoculations over a period of 4 weeks, of killed staphylococci. Twelve days after the last injection the prepared animals were tested by inoculation of living virus—some of the animals by subcutaneous injection and others by intravenous injection. All the animals reacted as if they had received no bacteria—they showed the same lesions as the control guinea-pigs.

This worker, believing that the period of twelve days from the last injection was not sufficient, made another series of experiments, in which he waited four weeks, before making the test inoculation. The results were the same.

In another series he injected the vaccine directly into the skin of guinea-pigs rather than under the skin, as in his previous experiments. After 4 to 6 days a local reaction developed, which consisted in redness and edema. The guinea-pigs were tested, without waiting for the inflammatory symptoms to subside, by inoculating a staphylococcus culture under the skin.

The majority of the prepared animals, after an inoculation of virus, only showed a slight increase of local reaction already existing, without any abscess forma-

tion. When a large area of skin was vaccinated, the reaction was less than in those guinea-pigs which were vaccinated by one single injection.

The conclusions drawn by the author were that the subcutaneous inoculation of killed staphylococcus cultures do not immunize, but intracutaneous injections protect rapidly and efficaciously against an intracutaneous or subcutaneous inoculation of virus.[8]

The researches of Frederick P. Gay were made with the streptococcus. The bacterium employed in his experiments was isolated from a case of empyema.

This streptococcus, when inoculated into the pleural cavity of rabbits, produced a purulent exudate. After a number of animal passages, the virulence of this streptococcus was increased, so that 0.001 cc. of the culture produced a fatal empyema in the rabbit. The same culture injected intravenously, produced a generalized septicemia. Following subcutaneous injection, an erysipeloid inflammation was produced, this being fatal in a certain number of cases.

Gay observed that by varying the method of inoculation, the rabbit which survived an intracutaneous injection of 0.1 cc. of culture, became resistant to a reinoculation of virus intradermally.

He noticed that this immunity was purely local, for the rabbit prepared by intracutaneous injection, was not vaccinated against an intravenous inoculation of virus. Inversely, the rabbit which survived an intravenous inoculation of virus acquired no immunity against an intracutaneous inoculation of virus. He found that the fatal dose of streptococcus following intravenous injec-

[8] C. R. Soc. Biol., Vol. LXXXIX, October 13, 1923, p. 828.

tion, was about the same as that which is lethal following injection into the skin.

Following the same line of reasoning, the American scientist made another important observation. The streptococci, injected into the pleural cavity, were capable of protecting the rabbit against an inoculation of virus into the pleural cavity, but were incapable of protecting it against an intravenous inoculation. The inverse was also true.

As a result of these experiments Gay concludes that there is a real local immunity in association with a general immunity.[9]

The experiments made by Carrère are particularly interesting, because of the organ on which he made his experiments.

This observer had the fortunate idea of studying the mechanism of vaccination on the eyes of rabbits and guinea-pigs. The immunity was produced with filtered cultures of staphylococcus, streptococcus and pneumococcus. These filtrates were injected under the conjunctiva, in the anterior chamber of the eye, or applied directly to the conjunctiva by instillation. The day following the vaccination, he inoculated living bacteria, either into the cornea, the anterior chamber, or into the vitreus. The effects of the vaccination were followed daily, in the vaccinated and non-vaccinated eyes. In all these experiments the right eye was vaccinated, while the left served as the control.

The study of the infection and immunity was particularly favorable, because of the simple histologic structure of the cornea, the absence of vessels, and the ease with which the lesions could be followed *in vivo*.

[9] Jour. of Immunology, Vol. VIII, January, 1923, p. 1.

In one series of rabbits and guinea-pigs, two drops of a staphylococcus filtrate were instilled into the right eye, every hour for five or six hours. At the same time, the same quantity of ordinary broth was instilled in the left eye. The following day the test inoculation was made. This was done, according to the nature of the experiment, by injecting the viruses into the cornea, into the anterior chamber of the eye or into the vitreus.

Following the inoculation of the staphylococcus culture into the cornea, a white infiltration took place about the point of inoculation in both eyes. After 24 hours the vaccinated eye returned to its normal aspect, while the control eye became the site of a virulent inflammation (chemosis, conjunctival secretion, ulcer of the cornea). The infection continued to develop and after about 15 days the anterior segment of the control eye was found atrophied.

The inoculation of the staphylococcus into the anterior chamber produced after 2 days, the same clinical symptoms in both eyes, but the vaccinated eye soon returned to its normal aspect, and after about five days no trace of the infection remained.

On the other hand, during this period the control eye became the site of a panophthalmitis, leading to a rapid purulent effusion.

When the inoculation was made into the vitreus, the vaccinated and non-vaccinated eyes reacted in the same manner, i.e., both produced a purulent infection. The immunity conferred by the application of the vaccine then stops at the cornea. The same type of experiments, made with the streptococcus and pneumococcus, gave identical results.

It is therefore possible to vaccinate the eye in 24 to 48 hours, by the instillation of filtered cultures or spe-

cific antivirus, into the conjunctival sac. This vaccination is sufficient to protect either the cornea or the anterior chamber of the eye against a severe infection. The immunity obtained is specific and is absolutely localized to the treated eye.

In another series of studies, Carrère introduced the antivirus under the conjunctiva, instead of instilling it into the conjunctival sac. The virus (staphylococcus, streptococcus and pneumococcus) was introduced 24 hours later, into the cornea, the anterior chamber, or into the vitreus.

The results indicate, that following this procedure, protection against a corneal infection or infection of the anterior chamber may be obtained, but not against an injection into the vitreus. In order to protect the vitreus it was necessary to inoculate the vaccine directly into the anterior chamber.

Histologic examinations made during the course of these studies threw some light on the mechanism of the immunity obtained. In the vaccinated eye, the inoculation of the virus is followed by a moderate leucocytosis of short duration, but the control eye shows a very severe reaction.

The writer calls attention particularly, to the integrity of the corneal cells in the vaccinated eye; the layer of cells is simply distended by the masses of leucocytes, while in the control eye the layer of cells is vacuolized and undergoes a real destruction. The bacteria entirely disappear after 48 hours in the vaccinated eye, while in the control eye they continue to develop.

In view of these microscopic findings, Carrère was led to believe that the local immunization is a simple desensitization of the receptive cells.

These researches, which are of great theoretical in-

terest, also have their practical importance. The difficulties that are encountered in ocular vaccino-therapy in man, are well known. It is then indicated, to substitute subcutaneous injections, by the direct application of antivirus to the eye, or in the eye.

As we will see directly, ocular therapy soon took advantage of these experimental facts.

II

Up to the present, we have discussed the experimental studies. We were particularly interested in knowing the mechanism of antistaphylococcic and antistreptococcic infection and immunity in laboratory animals.

It is now important to know how the staphylococcus and streptococcus act in man. Let us indicate the part played by the skin and mucous membrane in these infections.

The multiplicity of the localizations of these viruses in human pathology appears, at first sight, to argue against the idea of the elective affinity of these viruses for the skin and mucous membrane. But when we examine the facts closely, we note that this multiplicity of localizations is secondary, and that the mechanism of the primary infection is in reality, essentially quite like that which occurs in animals. All the infections, as varied as they may be, whether they are localized in the lungs, pleura, joints, kidneys or elsewhere, draw the virus most often from the same source; that is, the cutaneous covering. It is only after the staphylococcus or streptococcus becomes localized in the skin or mucosa that it may colonize in the organs. In man, as in laboratory animals, the primary lesion is almost always mucous or cutaneous. It is the cutaneous infection—by this we mean of the skin and mucosa—that dominates the etiology of the

greatest number of diseases in man, caused by the staphylococcus, as well as the streptococcus.

A staphylococcus that localizes in the bone marrow or the kidneys for instance, is always very serious. It may be objected that the question as to whether it is primary or secondary is unimportant.

This is true when we deal with an established infection; but in order to prevent an infection, the distinction is far from being unimportant.

If it be true, as we have just stated, that staphylococcus infection in man begins most often in the cutaneous tissues; and if it be true that the skin may be vaccinated, as our animal experiments indicate, then it is important to know, whether the staphylococcus infection is in reality secondary; that is, subordinate to a primary lesion of the skin or mucosa. For if it is, then cutaneous vaccination is indicated, so as to produce a barrier against the spread of the virus and in order to prevent new localizations.

Healthy man is amply covered with the staphylococcus. We find it on the surface of the skin, in the openings of the sebaceous and sweat glands, about the hair follicles at the point of immergence from the skin; we find it constantly on the surface of all mucous tissue: ocular, nasal, tonsillar and buccal. In short, under physiological conditions, the staphylococcus has a liking for the skin and its appendages. As long as the skin remains intact, man does not resent this symbiosis; but all changes, just as soon as the body resistance has been reduced, either by traumatism, intercurrent infection, or nutritional disorders.

This symbiosis rapidly turns to an infection. The staphylococcus, which was inoffensive up to the present, becomes pathogenic; it gives rise to furuncles, ecthyma

or impetigo, and as the process spreads, produces a lymphangitis, adenitis, phlegmon, etc.

Once a staphylococcus is localized it is not easily dislodged. We are not out of danger, even when an infection appears healed. When the initial lesion has disappeared and the tissues have returned to normal, a slight diminution in the general condition is sufficient to light up the focus. Under these conditions the vitality of the staphylococcus is restored and the virulence returns. This is what occurs in paranychia infections, folliculitis, mastitis, etc.

The staphylococcus, which is a normal host of the external auditory canal, is responsible for furuncles, a frequent and painful condition in this region. The middle ear, which is ordinarily aseptic, becomes infected by way of the Eustachian tube and gives rise to a suppurative otitis. It is also true that the staphylococcus in the sinus, is carried to the ocular mucosa by way of the lachrymo-nasal canal. Conjunctivitis most often, has a more direct origin; ocular infection is in a large measure fed by the staphylococcus, which is a normal host of the conjunctival sac. Blepharitis, styes, furuncles of the eyelids, phlegmons of the eye, abscesses of the cornea, phlyctenular keratitis, dacryocystitis, are most often caused by the staphylococcus. The staphylococcus does not scorn any of the mucous tissue. Localized normally in the vagina, it often ascends, and under favorable conditions, may be found in the uterus and adnexa. It may then produce bartholinitis, vulvovaginitis, metritis and salpingo-ovaritis.

The staphylococcus, which under ordinary conditions resides in the nasal cavity, may descend because of an inflammatory condition in the pulmonary tree, and create disturbances there. It either acts alone or in

association with other bacteria which are already present.

The various diseases just reviewed, are most often of a mucous or cutaneous origin. This local origin appears to be less clear in osteo-articular, renal or cardio-vascular localizations.

In osteomyelitis, the primary cause is often a furuncle, an abscess or simply a lesion of the skin. Infectious endocarditis may also begin with a staphylococcus infection of the skin or mucosa. Even in pyemias, with miliary abscesses of the kidneys, one almost always finds a break in the skin to begin with, which has become infected with the staphylococcus. It may be a furuncle of the face or neck or an angina, which precedes the pyemia.

In short, whatever clinical form the staphylococcus infection may take, from a simple herpes to an infectious endocarditis, one often finds the source to be an initial cutaneous infection.

The staphylococcus, which is accustomed to live in symbiosis with the muco-cutaneous tissue, penetrates the lymphatic vessels of the skin, just as soon as it has sustained the slightest injury. Having entered the lymphatic current, the staphylococcus creates a lymphangitis. Upon reaching the glands it meets the phagocytes, where it is digested and destroyed. After a slight reaction, the lymphatic glands returns to their normal aspect, or the microbe gains the upper hand, passes the glandular barrier, enters the thoracic duct, passes into the subclavian vein and finally reaches the general circulation. Once having reached the blood stream, the staphylococcus rarely grows in it. Most often it localizes in the organs. Depending upon the resistance met with

in the organs, and following its affinity, the micro-organism localizes in the bone marrow, in the endocardium or in the kidneys. In this way diseases are created, whose gravity contrasts with the benign lesions of the skin or mucous membrane noted at the outset.

The streptococcus has many points of resemblance to the staphylococcus. The streptococcus may grow in all the organs. The prognosis is ordinarily more serious than in staphylococcus infections. The streptococcus gives rise more readily to septicemia. While in staphylococcus infection, the pus is very thick and creamy with a tendency to localize, the contrary is true for the streptococcus. With this bacterium the pus contains the debris of destroyed tissue and the disease begins with symptoms, that at once react upon the entire organism.

The localizations of both bacteria are about the same. The streptococcus, on entering the circulation may give rise to lesions of the vessels (arterites, phlebitis); of the heart (endocarditis); of the organs (nephritis, peritonitis, hepatitis). We know the part that the streptococcus plays in puerperal and wound infections. In those conditions it is the cutaneous or mucous tissue which is infected at first.

We may therefore state, without mentioning all the clinical facts, that in the majority of instances in man, the mechanism of infection is the same as that observed in laboratory animals. Cutaneous infection is the basis of most staphylococcus and streptococcus infections.

In the preceding section devoted to experimental data, it was shown that the failure of vaccination in the guinea-

pig was due to the method of vaccination employed, rather than to the nature of the vaccines used.

We learn from the above studies that we need not depend upon the production of antibodies in obtaining immunity. In order that vaccination may succeed, it is of importance that the receptive tissues be involved in the process of immunization. The immunization of the latter is more important than the attempt to increase the quantity of antibodies in the blood.

With this idea, the method to follow in practice, should not be an attempt at antibody production, but vaccination of the tissues that are menaced by infection; the cells of the skin, the mucosa of the mouth, of the eye, of the uterus, the pleura, the bone, the intestinal wall, etc.

When we are confronted with a streptococcus septicemia, for example, where the portal of entry of the bacteria is unknown, or where the principal focus of infection is inaccessible to a wet dressing, the bacterial filtrate may be injected directly into the circulation. The antivirus may limit the focus, by coming in contact with healthy cells that are still intact, but are being menaced. It may stop the source of infection when it is localized in an internal organ, and so prevent the virus from overflowing into the circulation. The favorable results of vaccinotherapy in man, as it is most commonly practiced, by subcutaneous injection, is due in our opinion, to the same cause. The success of the vaccine is not due to antibody production, but to the penetration of the antivirus into the cells which are about to be invaded, which the antivirus protects against infection.

Attempts at vaccinotherapy, based on the principles related above, have already been tried in medical and surgical practice.

We herewith cite a few examples, furnished by the physicians who treated the cases:

Furuncle of external auditory canal. Personal observation of Dr. W. Has suffered from recurring furunculosis in external auditory canal over a period of 8 months. Various methods of treatment tried, but furuncles always recurred. Last infection consisted of a number of small furuncles in the external auditory canal. Staphylococcus broth vaccine was applied locally in the form of wet dressings. Cure rapidly obtained. No recurrence of furuncles after a period of 3 months

Varicose ulcers. Case report by Dr. C. Man 78 years of age, has been suffering with varicose ulcers on both legs for about 20 years. Each ulcer about 3 inches in diameter. Patient developed symptoms of phlebitis and was put to bed. A broth vaccine was applied to each ulcer by means of a wet dressing. After 8 daily applications, the ulcers healed and closed Patient up and about for some weeks after treatment and ulcers still closed.

Furunculosis. Case report Dr. K. Number of furuncles on neck of 2 months duration. Various methods of treatment of no avail. At time of examination 17 small and 4 large furuncles found on neck. Patient in great pain Could not sleep or be moved. Pustules opened and staphylococcus broth vaccine applied. Pain stopped immediately. Next day inflammatory symptoms began to diminish Violet color of skin disappeared and glands decreased in size. After 3rd day of treatment core of furuncles found in dressings. Healed rapidly.

Furunculosis. Case report of Dr. S. Patient came to see physician on December 4, 1924. Complaining of furuncles on neck. Wet dressings applied until December 8, but of no avail. Furuncle opened by incision. Opening admitted two fingers. Broth vaccine applied by wet dressings. After 24 hours core was found in the dressing and wound appeared clear and clean. Wound closed in 13 days

Furunculosis. Case report of Dr. T. Woman 60 years of age, diabetic. Large furuncle on neck between shoulders. Furuncle opened by incision and usual wet dressings applied. 20 days later, 2 new furuncles developed on the back in the lumbar region. Staphylococcus broth vaccine applied to these 2 new furuncles. Both of these furuncles healed 15 days sooner than the first, which was treated in the ordinary manner.

Psycosis. Case report of Dr. N. Patient suffered with recurring psycosis barbae of mustache, which appeared twice a year. Ordinary methods of treatment did not help Staphylococcus broth vaccine applied by means of a wet dressing, after previous removal of the hair. A definite cure rapidly obtained.

Folliculitis of the inferior lip Case report of Prof. Bernard Ballet, Gaz. Med. Nantes, August 15, 1924. "In 1919, skin of upper lip became invaded with a squamous lesion, which shed small pieces of dry skin. Had mustache at time. From 1919 up to few weeks before this report, condition remained stationary, in spite of applications of various ointments and antiseptic lotions. In 1921 underwent complete epilation of mustache by X-ray which led to a cure of 6 months' duration

In January, 1924 made application of wet dressings of a commercial broth vaccine.

When we saw the patient for the first time, the upper lip, in the entire area formerly occupied by the mustache, was covered with a yellow scab. When the corner of the scab was lifted with a pair of forceps an exudate was found on the epidermis. No mycelia were found in the removed hair.

Culture of pieces of hair on various media revealed no trichophytosis, but a pure culture of staphylococcus aureus was obtained

Wet dressing of a staphylococcus broth vaccine was applied to the area. No other treatment was employed. We first employed a solution of an agar culture in sodium hydroxide, following the technic of Maute. After this we prepared a broth vaccine. The culture was grown in broth for 8 days and then freed of bacteria by filtration; it only contained the soluble substance derived from the bacteria.

The patient made 13 nightly applications with this wet vaccinating dressing and removed it in the morning. In a few weeks the result was surprising, for the crusts did not form any more, and the skin which had been indurated and covered with nodules, became soft."

We are dealing with a staphylococcus infection, which resisted all therapeutic measures for five years, and was finally cured by a bacterial dressing.

Boil on the chin. Case report of Dr Bass and Dr. Levy. Miss Marguerite R suffered from an infection of the chin Examination revealed a large boil in the center of the chin.

The temperature was elevated (The evening preceding the examination, it was 40.5). The patient was delirious. Complained of pain

in the head and neck and difficulty in chewing. The lower gums were greatly swollen.

On the evening of November 14th a dressing soaked in a mixture of *staphylococcus* and *Streptococcus* broth was applied. The following day the general conditon remained the same. Temperature still high; had a restless night.

November 16th, liquefied pus found spread over the dressing. The inflammatory area decreased in size and the pain was less intense.

The dressings of broth vaccine were continued twice daily until November 19. At this time chin had returned to normal.

Osteomyelitis of left femur. Case report of Dr. Bass, Dr. Soupault and Dr. Brouet. Surgical Clinic of Professor Hartmann at Hotel-Dieu. Albert, 27 years old. Injured in right thigh by gun-shot wound in 1915. Recently, some pain in right leg with difficulty in walking. Swelling soon appeared and patient confined to bed. Temperature between 38 and 39. General condition bad.

On March 3, at the site of the old lesion, two abscesses appeared, which were followed by a swelling of the inguinal glands. Femur enlarged. Temperature reached 40°.

March 14, wound was opened and drained.

March 17, pus removed for examination and preparation of broth vaccine. Staphylococcus found.

March 27 the first dressing of an autoantistaphylococcus vaccine was applied. Patient left hospital on March 31. He returned for dressings for a few more days, but wound closed rapidly.

Pyodermia in the new-born. Case report of Dr. Ribadeau-Dumas and Dr. Debary—unpublished. Infant George Z, born June 30, 1924, brought to the clinic on September 18, 1924, for fever and multiple subcutaneous abscesses, which had been treated in a maternity clinic for about 6 weeks. The abscesses recurred continuously.

On arrival in the clinic many abscesses were found, which were in various stages of development; some beginning and some suppurating. They were disseminated over the body and head. During the first four days at the hospital the temperature rose above 40°, and the weight dropped almost 300 grams. Child had a slight diarrhea. After an examination of the pus, the abscesses were dressed with the antistaphylococcus filtrate. The abscesses healed in 4 or 5 days.

September 29. Temperature returned to normal, and the child had gained weight.

October 27. Child well, having gained 1,200 grams during the stay in the hospital.

Pleural fistula. Case report of Drs. Bass, Soupault and Brouet. Surgical clinic of Professor Hartmann at Hotel Dieu. Patient 44 years, underwent amputation of right breast for epithelioma on July 12, 1923. A few days after the operation symptoms of pleurisy developed.

July 25. Rib resected.

July 30. Two Carrel tubes inserted, for irrigation with Dakin fluid. This treatment continued during entire month of August. Culture revealed *streptococcus.* Putrid discharge continued. Temperature varied between 38° and 39°. General condition bad.

We were dealing with a case of pleural fistula. Rib had been resected one month previously, without much improvement.

On August 30, anti streptococcus broth vaccine was instilled in the pleural cavity, by way of the fistula (60 cc. at each injection).

After 20 days of this treatment, the temperature dropped, the opening closed, and the general condition improved.

Streptococcus pleurisy. Service of Professor Rieux at Val-de-Grace. Jean, soldier. Entered military service May, 1924. Became ill about June 8, 1924. Complained of increasing pain in right chest for past 4 days.

On admission showed general appearance of infection. Temperature irregular and slightly elevated. Examination showed signs of fluid at right base.

June 16. Exploratory puncture of right pleural cavity revealed a cloudy liquid, which on examination showed polynuclear leucocytes and *streptococci.* 200 cc. of antistreptococcus broth vaccine injected into pleural cavity.

June 18. Pulse and temperature improved. Puncture revealed 100 cc. of a cloudy serous liquid. Thirty cubic centimeters of broth vaccine injected.

June 20. Exploratory puncture negative.

June 21. Puncture revealed purulent liquid, which on examination showed no bacteria. Fever had completely dropped—improvement marked.

June 27. No temperature, no pain. Diminished breathing at right base. Exploratory puncture negative.

June 30. X-ray showed right diaphragmatic movements diminished; right base slightly cloudy, but no fluid present.

July 14. X-ray showed right base slightly cloudy, but right hilus absolutely clear.

Patient left hospital on July 24th for convalescence.

Puerperal infection. Case report of Drs. Levy-Solal, Simard and Leloup, C. R. Soc. Biol., February 23, 1924, p. 483. Primipara, premature rupture of membranes during night of January 11–12. Complete dilatation on January 14.

Delivery by forceps.

January 15, 6 p m. Temperature 38°, pulse 122, face drawn, tongue dry.

January 16, a.m. Temperature 38°, pulse 136, subicteroid appearance marked. Afternoon temperature 39.4°, pulse 144, small and intermittent. Condition serious Intra-uterine packing with anti-streptococcus broth vaccine.

January 17. Morning temperature 39.2°, pulse 140, intermittent. Profuse sweating during day. The evening temperature 38°, pulse 140. Second packing with broth vaccine.

January 18. Temperature 37.5°, pulse 120 and regular. In the evening, temperature 37.9°

January 21. Temperature and pulse normal. During succeeding days patient improved and left bed on February 6.

Bacteriological examination of lochia and of uterine culture revealed many short chained *streptococci.*

We were dealing here with a case of streptococcus puerperal infection, where treatment was begun on the fourth day after the delivery It was shown that the curative action of a filtered streptococcus broth culture was definitely beneficial

Puerperal infection Case report of Drs Couvelaire, Levy-Solal and Simard, Bull. Soc d'Obstetrique et de Gynecologie, No. 4, Meeting of April 7, 1924, p 232. Case 1. This woman, the day following an artificial delivery, had a rise of temperature to 40°, chills, pulse 120, and a leaden color.

The patient remained in a serious state during the following two days On each of the three succeeding days 80 cc. of antistreptococcus serum were injected. No change was noted—the pulse remained rapid, color leaden, and tongue dry. An intrauterine tampon of 80 cc. of antistreptococcus broth vaccine was made. Four dressings were made at intervals of 24 hours. After this a definite improvement was noted.

Case 2. The same method was applied to a second case. The temperature was normal for two days following the delivery, but on the third day there was a rapid rise to 39°, then 40.5° and on the 4th day 41° General condition serious, pulse 130 Uterus enlarged and

painful Appearance of patient indicated a serious infection. During following three days patient received 80 cc. of antistreptococcus serum by subcutaneous injection. General condition remained the same. During the following three days, daily uterine applications were made with a wet dressing soaked in antistreptococcus broth vaccine. Following this the grey aspect of the cervix rapidly changed and the general condition completely improved. Convalescence continued and patient recovered.

Examination of the lochia in both these cases revealed the presence of *hemolytic streptococcus*.

Puerperal Infection. Case report of Dr. Levy-Solal and Dr. Simard. Presse Médical. July 22, 1925, No. 58, p. 977. Case I. 35 years. Para II. Normal gestation. Large fibroid of uterus. Went in labor May 5, at 9 a.m. At 6 p.m. dilatation complete Forceps applied because of feeble fetal heart. Child apparently dead at birth but began to breathe after some minutes. Plancenta delivered without interference.

May 6. Temperature 37.3 pulse 84; p.m., temperature 37.5, pulse 88; during night violent chill

May 7 9 a.m, temperature 40, pulse 140, face drawn, chill. Examination showed nothing abnormal. Uterus at level of umbilicus. Lochia without odor. At 4 p m. temperature 41°; pulse small and rapid, 154, chill. Intra-uterine packing with broth vaccine. No membranes found on examination before making intra-uterine dressing. At 2 a m. patient had violent chill and was almost pulseless. 10 cc. of camphorated oil given in 2 injections.

May 8. Temperature 39, pulse 120, stronger General condition better No new chills. Generalized urticaria. p.m., temperature 38.2, pulse 110. Uterine tampon removed and another soaked in the broth vaccine applied. Patient slept.

May 9. Temperature 37.2, pulse 98. New urticarial eruption p. m., temperature 36.9, pulse 88. Third dressing applied.

May 10. Temperature 36.9, pulse 80. Nothing abnormal. Patient left bed on the twentieth day, in excellent condition.

Case II. 27 years. Part I. Normal delivery on March 12 No temperature during first 2 days. On third day, temperature 40.5°, pulse 160, chills and delirium. Blood culture taken, which subsequently became positive. Uterine tampon with broth vaccine applied. On the 16th a.m, temperature 38 5, pulse, 110; p m. temperature 39, pulse 120. Second uterine dressing with broth vaccine made.

On 17th, temperature 37.5, pulse 100. General condition good; p.m., temperature 38.2, pulse 100, third dressing made.

On 18th, blood culture negative. Temperature 36.8°, pulse 100. Patient sat up on 18th.

Case III. 24 years. Part II. Spontaneous delivery at home, no physician present. Evening of second day, temperature 39.5, pulse 120. General conditon bad, face drawn. Blood culture negative. Dressing with broth vaccine applied.

March 3, following day, temperature 38.2, pulse 106; p.m., temperature 38.6, pulse 126. Second wet dressing made. General condition good.

March 4. Temperature, a.m. and p m., 38, pulse 120 and 88.

March 5. Temperature, 38, pulse 84; p.m., temperature 37.2, pulse 80. Condition good. Patient recovers.

We herewith cite five cases of ocular infections, that were treated according to the principle of local vaccinotherapy, by instillation and washing of the lachrymal duct with specific dressings (Carrère, Bull. Soc. Ophthalm., Paris, March, 1924, p. 106. Soc. Sciences medic. et biolog. de Montpellier, March 14, 1924).

Chronic catarrhal conjunctivitis. Double cataract. Purulent dacryocystitis. Examination of the conjunctival secretions revealed *staphylococcus albus* and a small thick bacillus. Cultures of pus from the duct revealed *streptococcus.*

Treatment was begun by instilling a mixture of staphylococcus and streptococcus broth vaccine into both eyes. Two drops were instilled four times a day.

The day following the beginning of the treatment the conjunctival secretions almost stopped and the congestion was greatly diminished. Pus was expressed from the right sac. The sac was first washed with sterile water and then with a streptococcus broth vaccine, taking care to leave a few drops of the liquid in the sac. The instillation of the vaccine was continued. After 24 hours the right eye appeared normal, the left eye being still slightly congested. Pressure on the sac revealed a serous liquid which contained a few drops of pus. On examination a few poorly stained bacteria were seen. Sac again washed with broth vaccine and instillations continued.

Cure was obtained in four days—that is, at this time the conjunctival

secretions had disappeared and pressure over the right sac only revealed a serous liquid, which was free of bacteria, by direct examination and culture.

A sound (No. 2) was passed. On the following three days, sounds (No. 2 and No. 3) were passed and washings with the broth vaccine were made.

Eight days later the right eye was successfully operated on for cataract. The evening before the operation and directly before it the broth vaccine was instilled into both eyes. Cure was permanent. The left lachrymal duct was open. The left cataract was removed one month after the beginning of the treatment.

Phlegmon of left lachrymal duct. Edema of cheek. Chemosis. Woman, 45 years. An incision into the duct revealed green pus. Curetted. Ordinary treatment followed which led to an improvement, but a fistula with local edema persisted; the patient refusing all surgical interference. Examination of the pus showed *staphylococcus aureus*. A filtered broth culture of this bacterium was made and applied during the night by washings and wet dressings.

The following day the edema disappeared. Pressure over the sac revealed a sero-purulent liquid. Same treatment repeated. Three days later the secretion disappeared, the sac diminished in size and the fistula secreted a tear. On the fifth day of treatment the sac returned to normal and the cure was complete.

Injury to right cornea by piece of wire. Examination showed chemosis, with paracentral corneal wound about 2 mm. long. Edges of wound badly defined and infiltration into cornea found. Anterior chamber contained a slight hypopyon and iris was cloudy, contracted and reacted badly to light.

Culture from corneal ulcer revealed many *pneumococci*. Two drops of a broth vaccine obtained by the culture of pneumococcus types I, II and III, were instilled into the eye, five times a day.

The following day, the chemosis diminished, and the ulcer appeared better—the border was less infiltrated, the hypopyon was partly absorbed; iris appeared less opaque. The same treatment was continued, with the addition of atropin.

Twenty-four hours later, a circle of perikeratitis persisted, the corneal ulcer was improving. Only a very small surface was impregnated with methylene blue. The hypopyon disappeared, the iris was dilated. The administration of atropin was abandoned and only the broth vaccine was instilled twice. In the evening an ointment consisting of 5 per cent yellow oxide of mercury was applied.

The next day the cornea had returned to its normal transparent appearance. Methylene blue only took at one point. The anterior chamber was normal and the iris was contracted. Vaccine was abandoned and the yellow oxide of mercury was applied in the evening.

The patient was seen eight days later. The right eye was normal except for a superficial paracentral leucoma which marked the region of the lesion.

Ulcero-membranous blepharitis of both eyes. Child of 13. Condition had persisted for sometime, having resisted various local treatments. Microscopic examination and culture revealed *staphylococcus aureus.*

After carefully removing the crusts in the ciliary region, a dressing of staphylococcus broth vaccine was applied during the entire night.

The next day the edematous infiltration at the border of the lids was diminished. A marginal congestion persisted, with small crusts at the base of the eyelashes. In the evening, crusts were again removed and broth vaccine dressings were applied. In the morning the eyes appeared normal. Only a few crusts and slight marginal congestion remained All treatment was stopped.

The child was examined two weeks later. Right eyelashes showed a few follicular abscesses; left eye appeared normal. A wet dressing of the broth vaccine, prepared from the isolated bacterium, was applied. After 48 hours all traces of the local inflammation disappeared. Dressings were continued for four more days, and condition was regarded cured. After one month no evidence of the disease was noted. The physician advised general treatment of good food, air and arsenophosphate preparations.

Bilateral dacryocystis. Mrs. J., complained of bilateral dacryocystitis for past 15 years. She was treated, more or less regularly, by different oculists. Caustic washings, antiseptics, and catheterization were tried without success. When patient appeared for examination she had just been treated by catheterization. She complained that all treatments had failed and requested radical treatment, such as the removal of the lachrymal sacs On examination, evidence of a chronic catarrhal conjunctivitis was found, which was more marked in the left eye. In the internal angles on both sides there was a definite swelling. Pressure caused a serous liquid, containing yellowish-white flakes, to exude.

Bacteriological examination revealed the pneumococcus. Physician washed the sacs, with a pneumococcus broth vaccine, employing an Anel syringe which had a curved tube attached to it.

The next day the conjunctiva appeared less congested. For the first time in a number of years the patient awoke without finding the right eye closed. Pressure on the right sac revealed a serous liquid which contained a few white flakes. On the left side the contents of the sac were unchanged.

The following day the conjunctival congestion only appeared in the left eye. The physician regarded the right eye as bacteriologically cured. A preparation, made the previous day showed no bacteria. A sound (No 3) was easily passed. Catheterized and washed.

The next day the right eye appeared normal. Two drops of the broth vaccine was instilled on this side, while the left sac, which was still distended with serous liquid, was washed.

On the sixth day the right eye was completely cured. The left eye was regarded bacteriologically cured, for the preparation revealed no bacteria An attempt to pass sound (No 3) struck against a bony wall which produced a slight hemorrhage. The broth vaccine was applied.

After seven days, the right eye was regarded as cured. There was no lachrymal discharge. The ducts were open. The left eye showed a slight palpebral edema, which followed the attempt at catheterization the previous day. There was a slight lachrymal discharge. Pressure over the sac produced some tears. No treatment was made. The following day, the left eye appeared normal. A few drops of liquid could be expressed by pressure over the sac A sound (No. 3) was easily passed. The sac was washed with the broth vaccine. Patient returned four days later. Condition remained good. There was no further lachrymal discharge. Pressure over both sacs indicated no retention Argyrol passes easily.

One month later cure was regarded complete. The lachrymal discharge ceased and the canals were open.

In this case the usual medication applied more or less regularly for fifteen years, produced no results. Local vaccination led to a rapid cure in two weeks. The cure was not only organic, but functional as well.

It appears from these observations, that filtered cultures may be of service in medical practice. Wherever the localization of the infected cells may be—whether it be a furuncle, an osteomyelitis, a phlegmon, lymphangitis, pleural fistula, diseases of the eye, puerperal infec-

tion, or even in certain cases of generalized septicemia—the mode of action of the filtrate is the same. When applied by wet dressings to the infected tissues, it produces an influx of leucocytes, leads to the disappearance of the pathogenic agent, and facilitates the elimination of those cellular elements which retard cicatrization. By vaccinating the neighboring healthy cells, these filtered cultures succeed in surrounding the focus and so extinguish the inflammatory process.

The introduction of bacterial filtrates has enriched therapeutics. The discovery of bacteria that produce suppuration, gave birth to antiseptic dressings; we now know their advantages as well as their disadvantages. Then asepsis was born, and with it the aseptic dressing. With the latter, the danger of intoxications diminished, as the vitality of the anatomic structures was respected. Asepsis limited its field of action against infection, by relying more on nature, rather than a fight against the virus.

The specific dressing, with filtered cultures acts at the same time on the bacteria and the cells. It stops the multiplication of the former and activates the defensive properties of the latter. This dressing combines the advantages of the antiseptic dressing and the aseptic dressing, without having their inconveniences. Further, it has the advantage, which neither of the others have, of acting in a specific manner on the cells and the bacteria.

CHAPTER III

Dysentery

Affinity of dysentery bacillus for the intestinal wall. Inoculation of virus intravenously, anatomic lesions; bacteriological findings. Infection limited to the intestinal tract. Lesions following inoculation of dysentery endotoxin.

Subcutaneous inoculation of virus; anatomic lesions; bactetriological findings. Infection limited to the intestinal tract. Difference of virulence following method of inoculation, in reference to the receptivity for the intestine.

Apparent insensitivity of the gastro-intestinal canal, reasons. Effect of ingestion of killed dysentery bacilli; effect of ingestion of dysentery toxin

Difficulties of subcutaneous vaccination in man; attempts in Russia, France and Greece Sensitized vaccine.

Vaccination of laboratory animals; experiments of Dopter, Chvostek, Shiga. Personal experiments Variations of agglutination titre following vaccination by the mouth. Impermeability of the intestinal wall. Immunity following the ingestion of killed dysentery bacilli; immunity following ingestion of dysentery toxin Immunization of mice.

Nature of immunity obtained following vaccination by the mouth. Function of the intestine. Experiments of Ch. Nicolle and Conseil on man

Reports of the epidemics at Versailles and Petrograde. Studies of the Commission on Epidemics, of the League of Nations, results of vaccination on refugees at the Island of Hydra, at Phalère, and at the hospital Saint Georges, at Kokinia

While the affinity of the anthrax bacillus for the skin proved a surprise, that of the staphylococcus and streptococcus, was not unexpected. The affinity of the dysentery bacillus for the intestine, while still not demonstrated, appeared plausible to us.

The degree of this affinity could be best demonstrated by studying the results of various methods of inoculation

of the virus. In numerous experiments, we found that irrespective of the manner in which the virus may gain entrance into the body, the dysenteric lesions that are produced, are always the same. The bacteria are always disseminated in the body in the same way; in short, the mechanism of infection is always the same.

The affinity of the Shiga bacillus for the intestinal wall is so powerful, that it overcomes all obstacles in the way of distance. There are practically no barriers to prevent the bacterium from reaching its organ of choice. The Shiga bacillus always reaches its goal, in spite of the devious route it may follow, and in spite of the considerable loss of bacteria that may result en route. Sooner or later, the bacterium reaches the intestine.

Study of the infection, following intravenous inoculation of virus, brings forth many interesting facts; but of greater interest are the observations that follow subcutaneous inoculation.

Our experiments were mainly carried on with rabbits. The strain of Shiga bacilli employed, killed the animal after intravenous injection of $\frac{1}{10}$ of a 24 hour agar culture. Following this inoculation, a rabbit of average weight, died in one or two days.

The autopsy revealed the most marked changes in the intestine. Our attention was attracted to the marked vascular dilatation and transparency of the intestinal wall. The consistency of the intestinal contents, which was liquid in almost the entire length of the canal, was another fact that made us believe that the localization of the virus takes place in this organ.

This idea is confirmed, following microscopic examination, by demonstrating the elective distribution of dysentery bacilli along the intestinal canal. Even though the animal

dies 24 hours after an intravenous injection, the blood and organs remain sterile.

The urine remains sterile hence the urinary tract carries no bacteria. On the other hand, all or nearly all of the bacteria are carried to the intestine, from which place they are finally eliminated. The gall-bladder too, contains Shiga bacilli in large quantities, and in pure culture. Cultures made of the intestinal contents, from the level of the duodenum to the end of the small intestine, reveal a plentiful culture of Shiga bacilli. We were surprised to find these bacteria in pure culture, to the exclusion of all other associating bacteria.

Illustrative experiments follow:

January 22. Rabbit, 1,900 grams. Received by intravenous injection, $\frac{1}{10}$ of a 24-hour agar culture of Shiga bacilli.

January 23. Paralysis of posterior legs. Weight 1,670 grams.

January 24. Found dead in the morning.

Autopsy showed congestion of the intestinal wall. Gall-bladder full, distended; brown bile. Heart blood liquid. The small intestine, in practically its entire length, contains a greenish viscous liquid and a great deal of gas. The lower segment of the large intestine is empty. Cultures show:

Blood: sterile.

Urine: sterile.

Bile: Pure culture of Shiga bacilli.

Duodenum: Pure culture of Shiga bacilli.

Small intestine: At different levels shows, pure culture of Shiga bacilli.

January 14. Rabbit, 1,880 grams. Received by intravenous injection, $\frac{1}{10}$ of a 24-hour agar culture of Shiga bacilli.

January 15. Animal found dead in the morning.

Autopsy immediately performed, revealed: marked congestion of the intestinal vessels in its entire length. Gall-bladder not enlarged. The contents of the small intestine, from the duodenum down, contained a greenish viscous material. Nothing abnormal in the other organs. Cultures show:

Blood: Sterile.
Bile: Pure culture of Shiga bacilli.
Contents of small intestine: At different levels showed a pure culture of Shiga bacilli.

We see from these experiments, that even though the dysentery virus is inoculated directly into the circulation, it does not give rise to a septicemia. Although the infection is rapidly fatal, it remains localized to the intestinal tract.

The macroscopic picture is practically the same, when instead of inoculating living bacteria, the dysentery endotoxin[1] is inoculated intravenously. Judging from the characteristic changes that are found at autopsy, it appears that the elimination of endotoxin takes place, in a large measure, in the intestinal mucosa.

The distribution of the virus following subcutaneous inoculation is particularly striking.

When a large dose of living bacteria is inoculated subcutaneously into the rabbit, so that it dies in 2 or 3 days, we expect to find at autopsy that the virus has become generalized and that the animal dies of a septicemia. We are led to this conclusion because of the necessity of inoculating a large dose of bacteria subcutaneously in order to kill the animal.

The experiments show however, that this is not the case. Following injections under the skin, the dysentery bacilli remain localized in the cellular tissue for a certain period of time. Then, after becoming adapted to the new conditions of life, the bacteria produce a soluble derivative—a product of secretion or of autolysis. This substance, toxin or endotoxin, reaches the intestinal tract,

[1] For the method of preparation, see Annales de l'Institut Pasteur, Vol. XX, p. 304.

toward which it is attracted, by virtue of its specific affinity. Following this, the dysentery bacilli then move toward the intestinal mucosa. Nothing interferes with them during their long journey. This is so definite, that we may search for the Shiga bacillus in the blood, spleen, liver or urine of a rabbit dead of dysentery, and no evidence of them may be found. In order to find the bacteria we also look in the gall-bladder and intestine. It is here only, that we find the Shiga bacilli, often in pure culture, provided the cultures are made immediately after the death of the animal. We therefore see, that even though unexpected, the subcutaneous inoculation of the virus leads to an entero-infection.

As all the rabbits react in about the same way, we will only cite one illustrative experiment.

November 7. Rabbit, 1,550 grams. Received under the skin of the abdomen, 2½ tubes of a 24-hour agar culture of Shiga bacilli, emulsified in 8 cc of physiological salt solution.

November 8. Rabbit weights 1,400 grams A red edematous area is present at the site of injection.

November 9. Animal is sick, and during the afternoon the anterior limbs become paralyzed.

November 10. Animal is completely paralyzed. Weighs 1,270 grams. Animal being *in extremis*, is sacrificed.

At autopsy, we find at the point of inoculation, an edematous red area with an infiltration of a clear reddish liquid. The liver is congested and friable The organs appear normal, except the small intestine, where the vessels are markedly dilated. Swollen Peyer's patches are found in various places. The duodenum and the rest of the small intestine contain a viscous greenish liquid. The lower part of the small intestine contains a liquid material, which consists of desquamated epithelium.

Cultures of the blood, liver, spleen, and duodenum remain sterile. Cultures of large quantities of bile, give two colonies of Shiga bacilli Cultures made from various levels of the small intestine reveal many colonies of Shiga bacilli—most often in a pure culture. Culture of the subcutaneous tissue also reveals a pure culture of Shiga bacilli.

The intestinal contents are diluted with salt solution and passed through filter paper. The same is done with a quantity of urine. To the two filtrates, which are entirely clear, some highly agglutinating antidysentery serum is added. After about two hours at room temperature, a fairly heavy precipitate is formed in the two tubes. The intestinal contents and the urine then contain endotoxin, which is eliminated by the kidneys and the intestine.

The reaction in the rabbit takes place primarily in the intestinal tract, irrespective as to whether the animal is infected intravenously or subcutaneously, or whether it receives an injection of living or dead bacteria. The macroscopic and bacteriological examinations of the organs indicate this.

The receptivity of the rabbit, to be sure, is far from being the same, after intravenous and subcutaneous injection; but this difference depends less on the mechanism of infection than on the distance of the routes followed. The intravenous method is the most severe of all, because the bacteria have a direct route to the intestine. They also arrive at their destination without a great loss en route. On the other hand, the bacteria injected under the skin have a long route, for there are many detours; the bacteria may in a large measure be held up before reaching their destination.

This is why the subcutaneous inoculation is relatively inocuous. As we have shown, the virus inoculated under the skin remains localized for sometime, and there it liberates its endotoxin. This is evident from the edema which is present at the point of inoculation. The bacteria that still remain intact, escape from the cellular tissue and reach the intestine.

It is always the latter organ which is the receptive organ by choice, in dysentery infection. If, in order to obtain the same result, it is necessary to vary the dose

depending on the method of inoculation—it is essentially because the number of bacteria arriving in the intestine, varies with the route that is employed.

To summarize: When dysentery bacilli are introduced into the circulation, they do not develop uniformly in all the organs. In spite of the fact that they are introduced into the circulatory system, they finally reach the organ of choice. They are so obstinately directed toward the intestinal mucosa, because the intestinal cells bear a stronger attraction for them, than any other cell.

This affinity is particularly striking, when the inoculation is made subcutaneously. Do not the dysentery bacilli join their receptive cells, in spite of all obstacles—disregarding the distance which separates the skin from the intestine? All the bacteria do not arrive there; many of them are lost en route, but the strongest go directly to the gallbladder and the wall of the small intestine. The final lodging place, which they choose, is really elective, for we do not find them localized elsewhere; we do not find the Shiga bacilli in the blood or in the urinary tract.[2]

It appeared quite natural to find the bacteria in the intestine following intraperitoneal inoculation; for it is said that the barrier which separates the one from the other is easily passed. But it was unexpected to find the bacteria in the duodenum or the jejunum, after subcutaneous injection!

After obtaining these results, we directed our attention toward the study of the receptive cells in other diseases that resemble dysentery infection, such as the diseases of the typhoid-paratyphoid group.

[2] Annales de l'Institut Pasteur, Vol. XXXIII, p. 301. See also Violle. Bull. Acad Méd, December 6, 1921; Combiesco, C R. Soc. Biol., Vol. LXXXVII, October 21, 1922.

We could not help but think, that in spite of the relative resistance of the rabbit to the ingestion of the dysentery virus, that the intestine must be the receptive organ. We felt that the intestine must bear the same relationship to the dysentery bacillus, that the skin does to the staphylococcus and streptococcus.

It is said that rabbits react slightly, or not at all, after the direct introduction of dysentery bacilli into the intestinal canal. How then do we explain the receptivity for the intestinal wall, with the slight sensitivity after ingestion of the virus?

We felt that there must be many reasons for this—mechanical and chemical; for example, the action of ferments, the reaction of the intestinal contents, the presence of food in the intestine, etc.

By removing one of the reasons indicated, would it be possible to render the rabbit more sensitive?

During the course of our experiments, we were convinced that in the rabbit the ingestion of virus could be made toxic. In order to bring about this condition we must operate on fasting rabbits, so that the intestine is more or less free of its contents. We must also employ a strain of bacteria that is capable of producing a great deal of endotoxin. Our experiments have shown that the previous administration of bile acts as a means which permits the bacteria to come in direct contact with the intestinal wall.

When we first began our studies on experimental dysentery infections, we had the good fortune to use a very virulent and very toxic strain of Shiga bacilli. Because of this, we succeeded in producing the experimental disease without employing bile.

We will cite two experiments to make our point clear.

In one, we administered a fatal dose of dysentery bacilli by mouth; and in the other, a sublethal dose, which was sufficient to provoke an intestinal reaction.

Experiment I. Two young rabbits (1,360 and 1,490 grams) ingested killed Shiga bacilli. (Killed at 60° for one hour). Each animal swallowed $\frac{1}{4}$ of an agar culture of a Roux flask.

Five days later, the two rabbits were found dead. At autopsy, an intense congestion of the intestinal wall was found, with a bloody effusion in isolated areas. Nothing abnormal was found in the other organs. Blood and bile were sterile.

This experiment demonstrates that the rabbit is not insensitive to the ingestion of the dysentery virus as was previously supposed. Death of the animal may be obtained following ingestion of the virus, provided young fasting animals are used, and large doses of toxic bacteria are employed. At autopsy, the principal lesion is found in the intestinal wall, quite like the lesions found after an intravenous inoculation of living virus.

Experiment II. Two full-grown rabbits (2,100 and 1,980 grams) ingested $\frac{1}{8}$ of an agar culture of a Roux flask of heated Shiga bacilli, (60° for one hour.) Nothing abnormal was observed—the animals continued to eat and gain weight. After five days one of the animals was sacrificed. At autopsy, circumscribed hemorrhages were found in the intestine, which indicated a healing inflammatory process produced by the virus.

In this instance, where the bacterial bodies are slightly toxic, or where insufficient quantities are given, the animal does not die, but still it shows a reaction in its receptive organ. This may be observed by sacrificing the animal before the intestinal lesions have time to heal. It should be noted that these experiments were made with killed agar cultures, which were rich in endotoxin.[3]

The same intestinal changes may be produced with

[3] Annales l'Institut Pasteur, Vol. XX, p. 304, 1906

dysentery toxin; that is, with filtered broth cultures. Dumas and Combiesco[4] observed, that after rabbits ingested 10 cc. of this filtrate on four successive days, an edema and hemorrhagic effusion took place in the intestinal mucosa. A loss of weight, paralysis and death followed in one-half the number of the animals.

All these experiments then show that when dysentery endotoxin is taken by the mouth, intestinal and nervous lesions are produced, and that these lesions are comparable to those obtained after intravenous or subcutaneous inoculation of living cultures.

The action of the dysentery virus is always the same, irrespective of the mode of entrance of the virus into the body. If it is possible to vaccinate animals after the subcutaneous or intravenous injection of dysentery bacilli, then *a priori* we should be able to vaccinate them after taking the virus by the mouth.

We now come to the problem that interests the biologist and the epidemologist; namely, can the rabbit be vaccinated by the buccal route?

Before answering this question, we wish to make a short digression, in order to recall the results of antidysentery vaccination in man.

Bacillary dysentery is more frequent in the army than in the civil population. In spite of its frequency, we rarely resort to vaccination—not because we doubt the efficacy of the vaccine, but because we fear the toxicity which follows a subcutaneous injection. The toxic reaction is so severe that the vaccine becomes impracticable for use in man.

During the Russian-Japanese war, a number of attempts at vaccination on a large scale, were made in the

[4] C R. Acad. Sciences, Vol. CLXXV, p. 652, 1922.

Russian Army. This had to be abandoned, because the good results were out of proportion to the accidents observed. In France, an attempt at vaccination was made by Charles Nicolle in the South of Tunis. The method could not be popularized, because of the severe reactions obtained.

The same result was recently observed in Greece. The general reactions (fever, tachycardia, intense headache) and local reaction (pain at point of inoculation, abscess) were such, that even though the refugees were decimated by epidemics of dysentery, the attempt at vaccination had to be abandoned.[5]

The reaction may be greatly diminished by employing sensitized vaccines. In Germany, under the trade name of Dysbacta, a vaccine of this type was employed on a large scale. It consists of a combination of bacteria, dysentery toxin and antitoxin, (Boehnke). In spite of the advantages in regard to the reaction of the sensitized virus, antidysentery vaccination by subcutaneous injection did not become popular, and has practically been abandoned.

After this digression, let us return to the question of vaccinating rabbits by the mouth. We have already demonstrated two significant facts in our animal experiments. The first pertains to the definite affinity of the dysentery bacillus for the intestinal wall—and so makes us consider the disease as an entero-infection. The second is in reference to the ease with which we may produce dysentery symptoms in the rabbit after the ingestion of endotoxin.

We should mention, that at about this time, we found that the mouse may be vaccinated against paratyphoid B,

[5] Report of A. Gauthier, in regard to the vaccination campaign in Greece in 1923 (Commission on Epidemics—League of Nations).

after ingesting the virus.[6] So the idea occurred to us, to attempt to vaccinate against dysentery infection, by employing the same method.

We have had our predecessors—one always has. While we knew of the experiments of Dopter,[7] we did not know of those made by Chvostek and Shiga. These were brought to our attention by Calmette's review.[8] All of these experiments, which appeared at about the same time (1908–1909) are quite alike. They may be summarized as follows:

Dopter's experiments were made on rabbits and mice. One single meal of bacteria was unable to create an immunity. It was necessary to renew the ingestion, on two or three successive days. An immunity was then acquired, about the 10th or 12th day. The optimal vaccinating dose for the mouse, was five milligrams of bacterial bodies. Considering the dose, Dopter thought that it would be impossible to vaccinate man by this method; for it would have been necessary to give an enormous dose *per os*—15 grams of bacteria on three successive days.[9]

Chvostek's experiments were made on rabbits. This author fed to his animals living and dead bacteria. A 48-hour agar culture in a Roux flask, was emulsified in 50 cc. of physiological salt solution. Each meal consisted of 20 cc. of this emulsion. After a certain period, Chvostek proceeded to search for agglutinating, bacteriolytic and antitoxic properties. The immunity in the animals was tested by the intravenous injection of a fatal dose of dysentery toxin.

The author noted that the agglutinating power was

[6] See following chapter.

[7] Dopter, Ann Inst. Pasteur, Vol. XXIII, p. 677.

[8] Annales Institut Pasteur, Vol. XXXVII, p 900, October, 1923.

[9] C. R Soc. Biol , May 16, 1908; Ann. Inst. Pasteur, 1909, p 677.

practically nil, and that the bacteriolytic and antitoxic properties were far from constant. The animals usually demonstrated a fairly solid immunity, following the test inoculation. No parallelism could be drawn between the immunity and the degree of antitoxin in the serum.[10]

We will quote from Calmette's review, in reference to Shiga's experiments. "After ingestion of dead cultures of his organism, Shiga succeeded in immunizing his rabbits so well, that a subsequent intravenous inoculation of 0.1 cc. of dysentery toxin produced no intestinal disturbance. But these animals most often died after 3 to 7 days, with spinal symptoms—paralysis of the fore or hind limbs or of the bladder (dyspnea, and cardiac paralysis) while the control animals died in one or two days, with diarrhea, edema and hemorrhages in the intestinal mucosa."

While the ingestion of killed cultures, in Shiga's experiments, was unable to protect the rabbits against nervous symptoms, still it protected them against digestive disturbances; there was therefore, an intestinal immunity.

"Shiga has tried," we note again in the same review, "to vaccinate a number of subjects in various insane asylums, where dysentery occurred each year, and in localities where there were epidemics. The results obtained, he states, were very good."[11]

While studying the problem of vaccination by the mouth, the point that interested us the most was the mechanism of the immunity, rather than the possibility of its practical application. We did not try to determine whether we could vaccinate better by way of the mouth, than by subcutaneous injection. The problem that in-

[10] Wiener klin. Woch , April 2, 1908, p 453

[11] Calmette, Ann. Inst. Pasteur, Vol. XXXVII, p. 900, October, 1923.

terested us was, whether it was possible to vaccinate by the mouth, and if it was, on what the immunity depended. Is it the function of antibodies, phagocytes, or both? Is it a local affair, as occurs in antistaphylococcus and antianthrax immunity? Or does the mechanism of antidysentery immunity depend upon an unknown process?

A rabbit that has ingested living or dead dysentery bacilli, shows evidence of their passage through the body, for sometime thereafter. The bacterium leaves its imprint in the body—but where? Is it in the intestine or in the blood?

The agglutination reaction is the easiest means of demonstrating evidence of this passage in the blood; and so we began to search for it there.

The serum of three rabbits (1,680, 1,670, 1,830 grams respectively) was examined for the presence of agglutinins in regard to the Shiga bacillus, before and after the ingestion of four heated agar cultures (60° for one hour).

Before ingestion, the serum agglutinated the Shiga bacillus slightly, i.e., 1 to 25. After one ingestion of dysentery virus, the agglutinating titre was determined at various intervals. These are the figures obtained in the three rabbits: after 11 days, 1/100, 1/100, 1/50; after 18 days: 1/200, 1/100, 1/100; after one month: 1/50, 1/25, 1/25.

Another experiment was made on six rabbits (weights varied from 1720 to 1930 grams). We wished to determine the agglutination titre, after two feedings of dysentery bacilli, at eight-day intervals. The amount of bacterial bodies administered *per os* was at the limit of the fatal dose, for three rabbits died during the course of the experiment.

This is a short summary of the history of the three rabbits:

Rabbit 64. December 26. Weight 1,800 grams. Ingested ⅛ of a Roux flask of heated Shiga bacilli (60° for one hour) (40 cc. of physiological salt solution is added to the entire flask; 6½ cc. of this emulsion is removed and 25 cc. of ox bile and licorice are added)

January 3. Weight 1,780 grams. One-fifth of a Roux flask, prepared as above, given by mouth.

January 11. Weight 1,750 grams. Bled from ear vein. Agglutination of Shiga bacillus not present at 1:50 dilution (lower titre not made).

January 15. Weight 1,850 grams. Bled from ear vein. No agglutination 1:50 dilution (As normal serum occasionally agglutinated in 1:25 dilution, a lower titre was not made).

Rabbit 65. Died of dysentery intoxication, four days after the first ingestion of bacteria (⅛ of a Roux flask).

Rabbit 66. Died of dysentery intoxication, six days after the second ingestion of bacteria (⅛ of a Roux flask).

Rabbit 67. December 26. Weight 1,720 grams Received, by mouth, ⅛ of a Roux flask of heated Shiga bacilli (60° for one hour).

January 3. Weight 1,500 grams. Received by mouth, ⅛ of a Roux flask of heated Shiga bacilli (60° for one hour).

January 11. Weight 1,600 grams. Bled from ear vein. Serum does not agglutinate Shiga bacillus in 1.50 dilution (lower titre not made).

Rabbit 68. December 26 Weight 1,930 grams. Received by mouth, ⅛ of a Roux flask of heated dysentery bacilli (60° for one hour).

January 3. Weight 2,000 grams. Received by mouth, the same dose as before.

January 11. Weight 1,920 grams Bled from ear vein.

Serum does not agglutinate Shiga bacillus in dilution of 1–50 (lower titre not made).

January 28. Weight 2,070 grams. Bled from ear vein.

Serum does not agglutinate in 1:50 dilution.

Rabbit 69. Died of dysentery intoxication, four days after the first ingestion of bacteria (⅛ of a Roux flask).

We see that in the first instance, where the rabbits received one bacterial meal, an agglutination titre of 1 to 200 developed after a period of 18 days. After an interval of one month, the agglutination titre already showed a tendency to return to normal.

In the second case, where the rabbits received two bacterial meals at eight-day intervals, not a trace of agglutiin was found in the serum 8 to 12 and 25 days after the second meal.

What shall we conclude from this? It is shown that specific agglutinins develop after the ingestion of dead dysentery bacilli; but the interesting fact is, that after another ingestion of bacteria, an examination of the serum made during the following month, fails to show a trace of agglutinins.[12] It appears therefore, that after the first absorption of bacteria, the intestinal wall refuses to absorb any more. The intestine becomes impermeable, and as our experiments have shown, this impermeability is specific. We thought that we should search for the cause of the immunity in the intestine.

A corollary question now presents itself for explanation; namely, will animals resist a fatal dose of virus, after previous preparation by the buccal route?

Experiment I. Rabbit 24 1,880 grams. Received by mouth on June 1, ½ of a Roux flask of heated Shiga bacilli (60° for one hour). On June 20, $\frac{1}{10}$ of a 24-hour agar culture of Shiga bacilli was inoculated in the marginal vein of the ear.

Rabbit 25. Control. 1,880 grams. Received the same dose of living Shiga bacilli in the vein.

June 21. Rabbit 25 was found dead. Rabbit 24 was well and survived.

[12] This is what evidently happened in Chvostek's experiments, already cited.

Experiment II. Rabbit 26. July 23, 1918. 2,180 grams. Received by mouth, on July 12 and 15, $\frac{1}{10}$ of a Roux flask of heated Shiga bacilli (60° for one hour).

Rabbit 27 2,070 grams. Received by mouth, on July 12 and 15, at first 8 cc. of ox bile, and immediately after $\frac{1}{10}$ of a Roux flask of heated Shiga bacilli (60° for one hour).

Rabbit 28. Control 2,400 grams.

July 23. Eight days after the second ingestion of bacteria, the three rabbits were inoculated intravenously, with the same dose of living virus ($\frac{1}{10}$ of an agar culture). Of these three rabbits, only the control died the following evening. The two other animals permanently survived.

The results of these experiments show, that the ingestion of heated cultures confers an immunity against a fatal dose of dysentery virus when inoculated intravenously.

The same type of results have since been reported by Dumas and Combiesco.[13] These authors found that rabbits that had resisted the ingestion of filtered cultures, i.e., dysentery toxin, would subsequently resist four fatal doses of culture, when inoculated intravenously.

We also succeeded in vaccinating mice, by feeding to them bread soaked in a dead culture of Shiga bacilli. The mice remain without food for 24 hours, in order to facilitate their taking the bacterial meal. The bacteria and bread are then fed twice, at eight-day intervals.

The test inoculation is made about 10 days after the ingestion of the second vaccinating meal, by an intraperitoneal inoculation of $\frac{1}{20}$ to $\frac{1}{30}$ of a 24-hour agar culture. The control dies in less than 24 hours, while the vaccinated mice survive many days or indefinitely.

From all these experiments—which are unnecessary to multiply—the evidence is increased to show that the ingestion of killed dysentery bacilli, taken alone, or better

[13] C R. Acad. Sciences, Vol CLXXV, 1922, p. 652.

mixed with bile, in one or in two meals, is capable of conferring an active immunity. These conclusions corroborate the findings observed by Dopter, Chvostek and Shiga.

How do we explain this antidysentery immunity? We believe that the immunity depends upon the same mechanism, whether it be established after subcutaneous or intravenous injection of virus—or, as in our experiments, after the ingestion of killed cultures. It is essentially a local one. Everything leads us to believe, that following the ingestion of the bacterial bodies, a specific lesion is produced in the intestinal wall, which is a mild form of dysentery. When these lesions are healed, they lead to an antidysenteric intestinal immunity.

We believe that it is because of this erosion produced in the intestinal mucosa, that a part of the antigen given by mouth in the first meal, succeeds in passing the intestinal wall, and so gains entrance into the blood stream. This accounts for the fact that some antibodies, particularly agglutinins, are formed. Just as soon as the first lesion in the intestinal wall is repaired, the intestinal barrier closes and can not be passed. From this moment on the immunity is established. After this, the ingestion of bacteria may be repeated, but the dysentery antigen can not pass this barrier. The antigen being unable to enter into the circulation, no more antibodies can be formed.

When living dysentery bacilli are injected into the animal—even though inoculated under the skin—the intestine is the organ that shows the greatest reaction. It is here that we find the greatest lesion; and the greatest number of bacteria. Bacteriological and pathological examinations prove this.

When we attempt to vaccinate the animal by injecting

dead dysentery bacilli, the intestine always shows the result of the attraction which it exerts upon the bacteria. The protective action of the vaccine is measured, in this instance, by the quantity of dysentery endotoxin that reaches the receptive organ—namely, the intestine.

Even though we admit the possibility of an antitoxic factor in antidysentery immunity, we believe that the refractory state of the animal is mainly located in the intestinal wall. This immunity is local and takes place without the presence of antibodies—irrespective of whether the method of vaccination be subcutaneous, intravenous or buccal.

Until now, the rabbit and the mouse were the only animals on which dysentery experiments could be performed. But man has had to play his part too in contributing to the knowledge of this disease.

To Ch. Nicolle and E. Conseil[14] goes the full credit of having appreciated and carried out an experiment on two volunteers. On three successive days these volunteers ingested heated cultures of dysentery bacilli (heated at 72° to 75° for one hour). They took no food from the previous day to six hours after the absorption of the vaccine.

On the fifteenth and eighteenth days after the second ingestion, these two individuals, and two new control volunteers, swallowed cultures containing 10 millions of living Shiga bacilli.

The two control volunteers contracted dysentery, with Shiga bacilli present in the stools, while the two vaccinated individuals remained well.

This experiment, performed by Ch. Nicolle and E. Conseil, supplemented the facts observed in the rabbit, and

[14] C. R. Acad. Sciences, No. 11. March 13, 1922.

closes the experimental phase of the problem of vaccination. Now, it was possible to turn to practical application.

The following are certain facts observed during the course of various epidemics of dysentery. While they are not as definite as laboratory experiments, still they plead in favor of vaccination by the mouth.

During the month of July, 1923, a severe epidemic of bacillary dysentery occurred in a garrison at Versailles.[15]

The first three cases were fatal. The number of cases rapidly increased, and vaccination by the mouth was decided upon. This was only done, as an experiment, to a limited number of individuals.

The result of this attempt at vaccination are shown in the following table:

ARMY GROUPS VACCINATED	NUMBER OF PEOPLE	VACCINATED	NUMBER OF DISEASED AMONGST THE VACCINATED	NON-VACCINATED	NUMBER OF DISEASED AMONGST NON-VACCINATED
7th Company.	157	94	3	63	21
8th Company	162	92	6	70	22
9th Company.....	136	8	3	128	59
10th Company	131	90	3	41	32
11th Company. . ..	144	5	2	139	37
12th Company ..	123	14	1	109	37
16th Company ..	143	107	7	36	27
Co. s/c.....	136	136	17	0	0
	1,132	546	42 or 7 6 per cent	586	235 or 40 per cent

No individuals were vaccinated in the 13th, 14th and 15th Companies. In these three companies—484 men in all—there were 62 cases of dysentery. This number, added to the 235 cases in the other companies, made a total of 297 cases of dysentery among 1,070 non-vaccinated individuals, or 27.75 per cent.

[15] Anglade, C. R. Soc Biologie, Vol. LXXXIX, February 16, 1924, p. 395.

The following figures were obtained in the vaccinated groups. 7.6 per cent infected, among the men who had taken the antidysentery vaccine, and 40 per cent among the non-vaccinated men.

Major Anglade, who reported the results of the epidemic, states that the epidemic continued in the entire garrison, while it died out in the regiment that was first attacked.

About this time (July, 1923) a number of cases of dysentery occurred among the civil population in Petrograde.[16] An attempt at buccal vaccination was made, during the course of this epidemic, in several groups—especially in a refugee camp that contained 2,768 people, of from 65 to 85 years of age. Vaccinations were also made on 362 individuals, which represented the personnel of the Botkine hospital for infectious diseases.

The first case of dysentery occurred in the camp on July 13. New cases occurred subsequently, so that on the day when the vaccinations were completed— from July 31 to August 3—there were in all, 85 cases of dysentery or 3.07 per cent.

Of a total population of 2,768 individuals in the camp, 1,000 were vaccinated, while the rest 1,768, were not vaccinated.

From August 3, the last day of the vaccination, to September 15, the end of the epidemic, the following results were noted:

Among the 1,768 non-vaccinated—56 cases of dysentery.

Among the 1,000 vaccinated—12 cases of dysentery—of which 9 occurred during the 10 days following the vaccination.

[16] Antonovsky, C. R. Soc Biologie, Vol. CX, March 1, 1924, p. 564.

The morbidity among the non-vaccinated was 3.11 per cent, and among the vaccinated 1.2 per cent.

At the Botkine hospital, where all the dysentery cases of Petrograde were assembled, 257 individuals in the hospital were vaccinated by the mouth, and 105 acted as controls.

Of the 257 vaccinated, only one individual, a nurse, developed a mild attack of dysenery, fifteen days after the vaccination—or 0.4 per cent. Among the 105 non-vaccinated there were four cases of dysentery, or 3.8 per cent.

Of no less importance, was the attempt in Greece at vaccination on a large scale, which was carried on by the Commission on Epidemics of the League of Nations. This was made on 30,000 refugees, who were divided in many camps, and who lived under particularly favorable conditions for the occurrence of epidemics. The results obtained in the various camps were very encouraging. We will quote a few items from the report of this Commission.[17]

In May, 1923 a serious epidemic of dysentery occurred among the refugees on the Island of Hydra. There were 700 individuals, among whom there were 22 cases, and 3 deaths. All the refugees were vaccinated by the mouth. The epidemic stopped abruptly and completely.

All the refugees at a camp at Phalere were vaccinated in June, 1923. At the end of July the camp was taken over by the military authorities. The refugees that inhabited this camp, were relocated into a number of other camps. Three hundred and forty were placed in a nearby barrack

[17] A. Gauthier, Bull. Acad. Medec., Vol. XCI, January 15, 1924, p 72.

at Kokinia, where at the time, an epidemic of dysentery was raging. The new arrivals drank the same water that supplied the camp, which caused the epidemic at Kokinia and in the nearby camps. No cases of dysentery occurred among the 340 vaccinated individuals.

On August 4, 1923, 2,800 refugees returning from Cilicie, were interned at the hospital Saint-Georges. During the crossing, which lasted 7 days, there were 36 deaths from dysentery, and in the first 48 hours after disembarking, there were 44 more deaths from the disease. More than 200 sick individuals were admitted to the hospital.

A general vaccination among the refugees was carried out. Eight days later the epidemic completely ceased. Most of the refugees went to Salonica, and there no new cases developed among them.

The epidemic that occurred at the Camp of Kokinia, during the months of August and September 1923, almost has the value of a laboratory experiment.

More than 400 cases of dysentery occurred among this group of 4,800 refugees. The epidemic was serious, for there were 50 deaths when vaccination by the mouth was begun. Only two-thirds of the population were vaccinated, while the remaining one-third were unvaccinated. The epidemic immediately stopped among the vaccinated, but continued for many months among the non-vaccinated. Among this latter group, there were 194 new cases.

CHAPTER IV

The Typhoid Fevers

I. *The study of immunity in relation to the study of infection.* The slight virulence of the typhoid and paratyphoid bacilli offer a difficulty for this study. Vaccines that protect the guinea-pig against a typhoid peritonitis are ineffective against typhoid infection. Test of immunity.

Typhoid fever in the chimpanzee: its characteristics. Metchnikoff's experiments with new-born mice. Paratyphoid infection in rabbits; localization of virus in the intestine Analogy with localization in the chimpanzee

Diminishing natural immunity of the rabbit after ingestion of bile. Properties of bile. Sensitization of infected rabbits with bile. Anatomic lesions and distribution of virus, after infection by mouth and after intravenous inoculation. Mechanism of action of bile. Sensitizing action of bile in the guinea-pig. Permeability of intestinal wall after bile. Alimentary anaphylaxis in adult guinea-pig; ingestion of tetanus toxin in mice

II. *Experimental typhoid vaccination before the war.* Vaccination of anthropoid apes by the oral route. Vaccination of rabbits and mice by the same method.

Vaccination experiments after the war. Ingestion of paratyphoid virus in normal rabbits and in rabbits sensitized with bile. Immunization of sensitized rabbits with killed virus, taken by the mouth.

Mechanism of the immunity. Function of antibodies. Method of action of bile. Results at La Fleche. Results in Roumania.

Objections raised to the use of bile. Value of subcutaneous vaccination in man. Conclusions.

I

Whether we are dealing with the anthrax bacillus, the staphylococcus, the streptococcus, or the dysentery bacillus, there is a great parallelism between the method of infection by, and the immunization with, these bacteria. It

was following our studies on the distribution of the virus in the body, that we were led to the idea of cutaneous and oral vaccination. Let us then follow this principle in typhoid infection. We may be guided by the conclusions drawn from the mechanism of typhoid and paratyphoid infection in the animal, in arriving at a rational method of immunization against this disease.

The problem suggested, at first appeared very difficult, for the following reason. In dysentery infection there is an endotoxin which is easily liberated and which rapidly produces the well-known lesions. In typhoid infection in animals this does not occur. The endotoxin of the bacteria of the typhoid-paratyphoid group does not diffuse easily. The virulence of these bacteria is much less than that of the dysentery bacillus.

Laboratory animals can only be killed with the typhoid and paratyphoid virus after intravenous or intraperitoneal injection. These bacteria are only slightly virulent after subcutaneous injection. They are avirulent when taken by the mouth. Even those animals that are as susceptible, as the rabbit and the mouse, may resist enormous doses of these microörganisms after ingestion. Dead Shiga bacilli are toxic for the same animals—even when introduced by injection or ingestion.

We see then how difficult it is to follow the route of infection in animals infected with the typhoid and paratyphoid bacilli. How then may we devise a rational method of immunization?

In spite of the great progress made during the past years, the problem of antityphoid vaccination still remains unsettled. At the present time there are more than 20 different varieties of vaccines, but their protective value is far from being absolutely established.

All are of the opinion that of the various typhoid vaccines recommended the best is the one prepared with living bacteria. Unfortunately these vaccines may not be employed practically. All the other vaccines, made with dead bacteria, are much less efficacious. Of course, all these vaccines may protect the guinea-pig against the inoculation of one or more fatal doses of virus. All agree to this. But are these vaccines, which are able to protect guinea-pigs against a typhoid peritonitis, also able to protect man against typhoid fever? The entire question of typhoid vaccination depends on that point.

No one denies that in most instances, we are permitted to draw conclusions observed in animals for man. But then, it is essential that the disease presents the same form in both instances. It is not sufficient to employ the same bacterium in man and animal; but in order to justify this relationship, the bacterium should attack the same group of organs in both cases. The pathological and clinical pictures should be the same in both instances.

In order to illustrate this idea, we will take a concrete example. The cholera vibrion produces a cholera peritonitis or an intestinal infection in young rabbits, depending upon whether the virus be inoculated intraperitoneally or taken by the mouth. While it is easy to vaccinate against a cholera peritonitis, it is impossible to protect against a cholera intestinal infection.

In the problem of vaccination then, we must not be content to concern ourselves with the specific bacterium, but also with the organs wherein it localizes.

The bacterium is the only thing in common between a typhoid or paratyphoid peritonitis in the guinea-pig, which develops in 24 hours, and a typhoid fever in man, of a longer duration. Pathologically and clinically speaking these are two different infections. They differ

as does a cholera peritonitis from a cholera intestinal infection. The control of the vaccine in the guinea-pig, is then of little value.

In order to control the vaccines, should we depend upon the titre of bactericidal properties, complement fixation or other antibodies? Their importance, as far as a measure of immunity is concerned, has not been demonstrated, as we will soon see. A subject which has received bacterial bodies, subcutaneously or intravenously, can do no more than defend itself against this invasion. The body reacts to these foreign protein bodies, by producing antibodies, such as bacteriolysins, complement fixing bodies, or agglutinins. But we feel that it is incorrect to state that these antibodies are a proof of immunity.

The real experimental proof of immunity was first attained by Metchnikoff, who reproduced typhoid fever in the chimpanzee. This animal and the anthropoid apes in general, are the only animals that are sensitive to the ingestion of the typhoid virus.

Following the ingestion of cultures or of typhoid infected material, nothing abnormal is noted during the first few days. The monkey continues to live as usual; it eats and runs about as do its neighbors. After the fifth, sixth or eighth day, varying in different instances, we see the first symptoms of the disease appear. The period of incubation is usually longer when the infection is less severe.

The disease begins after the sixth or eighth day with an elevation of the evening temperature, which continues to rise on the following days. It reaches 40°C. and remains well above normal for four to eight days, showing only a slight morning fluctuation. This is the febrile

period. The temperature then falls progressively, so that after about three days, it has reached normal.

During the height of the febrile period, typhoid bacilli are always found in the blood. The serum agglutinates, in dilutions from 1:50 to 1:400. The stools are most often diarrheal in character.

Most often the animals recover. Because of its benign character and its evolution, the disease reminds us of a mild typhoid fever in infants. The chimpanzee rarely dies of this typhoid infection. When it does, at autopsy we find a pure culture of typhoid bacilli in the liver, spleen and lymphatic glands. The Peyer's patches are greatly enlarged and congested. These lesions are particularly marked at the level of the ileo-caecal valve.

The experimental infection in the anthropoid ape, which differs from infection in the guinea-pig, but resembles that in man, may then be employed in determining the efficacy of an antityphoid vaccine. The experiments made by Metchnikoff and ourselves along these lines, in chimpanzees, orang-utangs and gibbons have been reported. We can not review them here, but further on, we will recall one of these experiments because it has a direct bearing on our subject.

While experiments on anthropoid apes are of the greatest importance, still it is prohibitive to work on a large scale with these animals in our latitude. It was therefore necessary to employ a more readily available animal. Metchnikoff attempted to do this. While alone in his laboratory at the beginning of the war, and desiring to make his contribution for the common good, he decided to return to the study of paratyphoid B infections.

He began by employing white mice. He desired to reproduce the disease following ingestion of the virus,

in the hope of being able to employ this animal in testing antityphoid vaccines.

Led by his old experiments on cholera in young rabbits, he began to employ young suckling mice. At the very beginning he succeeded in producing a fatal paratyphoid infection in new-born mice, after feeding them with the cultures. But after this first success, he soon noted failures. Had the cultures lost their virulence, as so often happens? He was unable to repeat his experiments.

Man may only contract the disease by the oral route. In laboratory animals, on the contrary, this is the least efficacious method of all. We have given enormous doses of living bacteria by mouth to rabbits, mice and different species of inferior monkeys, without producing the least disturbance.

We then tried to decrease this resistance by various methods. When the rabbit is injected intravenously with the typhoid or paratyphoid virus, we obtain either a generalized infection, without any definite characteristics, which kills the animal in less than 24 hours, or a subacute infection, which lasts three or four days or even longer. In the latter instance the blood culture is positive. It is interesting to note that the bacteria, responding to their affinity, lodge in places where we least expect to find them.

If at autopsy, we not only culture the blood, but also examine all the organs, we will be surprised to note the distribution of the bacteria. The cultures will show, that there are very few colonies in the blood, the typhoid and paratyphoid bacilli may be found in great numbers in the gallbladder, the duodenum, and the jejunum. Often they are found in the entire length of the small intestine.

When the death of the animal takes place slowly, the bacteria are more numerous in the intestinal contents. If the disease is prolonged for more than two or three days, we are certain to find a pure culture of paratyphoid B bacilli in the bile or in the contents of the small intestine.

The lesions in the gallbladder and in the intestinal wall are particularly striking—because the other organs are quite normal. The macroscopic examination makes us feel that the typhoid-paratyphoid infection does not take place in the blood, but principally along the intestinal and biliary tracts.

This elective distribution of the virus, and localization of the lesions produced in the rabbit, following intravenous inoculation, recalls the picture found in the chimpanzee, following ingestion of the virus.

Instead of chimpanzees, we may then employ rabbits for our experiments, provided one inoculates the typhoid and paratyphoid virus intravenously.

Having taken into consideration this elective localization of the virus in the intestine, we felt that we should repeat our old experiments, in an attempt to infect the rabbit following administration of the virus by mouth. Of course we could not think of administering the virus *per os* alone. The failures of other workers, as well as those of Metchnikoff and ourselves, were known. But, we questioned, what would happen if we succeeded in reducing the natural immunity of the animal by some laboratory artifice? We already know of certain methods which do this, such as reducing the temperature of the animal, the injection of a weak acid, or a concentrated solution of salt, or the injection of a slightly virulent bacterium, etc.

Beginning with the idea, that the point of predilection of the paratyphoid virus is the intestinal wall, our first idea was to try to modify the intestinal wall in some manner. Would we not be able to weaken the intestinal barrier, by producing an imperceptible break in the mucosa? Would not this break permit the bacteria to gain access to the wall, and eventually permit them to pass it? We thought of employing ox bile, in order to bring this about.

Our experiments have shown that rabbits that have ingested ox bile, became sensitive to the paratyphoid B bacillus. The ingested bile reduces, in a large measure, the natural resistance of the rabbit to paratyphoid infection.

Bile has the property of increasing the absorption and assimilation of material in the intestinal tract. Fatty substances are digested and absorbed better after taking bile. Albumins, precipitated in acid solutions, are redissolved in an excess of bile. The mucinase of the intestinal wall acted on by bile, prevents coagulation of the mucus and keeps it in solution. Bile activates the intestinal cells to secrete their digestive ferments and by virtue of their zymosthenic action, it even activates certain ferments (Roger).

Bile is an excellent cholagogue; when given by mouth to rabbits, it stimulates an active secretion of bile. Following this abundant secretion, an intestinal desquamation appears, and the paratyphoid bacilli in the intestinal canal may then easily reach the intestinal wall. When the bacteria are alive and virulent, a fatal paratyphoid infection develops, which begins in the intestine.

We have already called attention to the resistance of rabbits, to the ingestion of the typhoid and paratyphoid

virus. We have been able to feed as much as one-half to a whole Roux flask of an agar culture, without producing any other effect than a slight loss of weight.

When bile is ingested, previous to the administration of the paratyphoid virus, the picture is different. In this instance, with a much smaller quantity of culture, a fatal infection can be induced in a few days. The disease does not begin at once. After an incubation period of from one to four days, during which time no evidence of infection is present, the stools gradually become diarrheal in character. The animal remains quietly in the corner of the cage, and takes very little food. Soon after, the animal begins to go down hill—for in a few days it loses from 300 to 400 grams. From time to time the animal cries out, the temperature drops, and the diarrhea becomes so profuse that the posterior limbs are practically bathed in fecal material.

The disease lasts two or three days more and terminates with a sharp drop in temperature. Occasionally the cachexia is slow, and in this instance, the animal presents a deplorable state for 12 or 15 days.

The striking picture at autopsy is the condition of the small intestine. It is greatly congested, partly transparent and filled with a green viscous liquid, which contains masses of desquamated epithelium. The wall, which is thinned out by the desquamation, reveals swollen Peyer's patches. We are struck by the increase in size of the gall bladder—it contains as much as 8 cc. of clear greenish blue bile, or it is completely colorless and cloudy.

Cultures made from the bile and blood reveal a pure culture of paratyphoid bacilli. This is particularly the case when the disease has been of short duration. The stomach contents are sterile. Cultures made of the small

intestine at various levels, reveal an abundant number of paratyphoid colonies. It is not rare to find the bacteria in pure culture. Paratyphoid bacilli may also be found in the large intestine, but the number of colonies decreases as we reach the terminal portion.

The following is a record of one of these infected rabbits.

Rabbit 1,195 grams.

November 23. 9 a.m, received by mouth, 5 cc. of ox bile. 4 p.m., temperature 38.5°.

November 24. 9 a.m., temperature 38.7°; received by mouth 3 agar cultures of paratyphoid bacilli, emulsified in milk. At 3 p.m., 5 cc. of bile by mouth. At 4 p.m., temperature 39°.

November 25. Diarrhea since morning. At 9 a.m., temperature at 38.2°. At 4 p.m., temperature 37.8°.

November 26. Profuse diarrhea. Cage had to be cleaned several times during the day. At 9 a m., temperature 37.2°. At 4 p.m., temperature 36.4°.

November 27. Found dead. Autopsy: small intestine congested in almost entire length; a slightly cloudy liquid seen through the intestinal wall; the gallbladder is greatly distended; spleen small; nothing abnormal otherwise.

Cultures made on litmus lactose agar, of heart blood and bile, reveal characteristic paratyphoid colonies. Cultures of stomach and bladder remain sterile. Cultures of the contents of the small intestine, taken from various levels, reveal a pure culture of paratyphoid bacilli or an association with *B. coli*. Cultures from the coecum and appendix, on litmus agar, reveal as many blue as red colonies. In the colon and terminal portion of the intestine, paratyphoid bacilli are rare. Here we find a great number of colon bacilli.

Ox bile taken without any bacteria is inoffensive in the dose indicated. The sensitization of the animal is not due then to the toxic action of the bile. The action

of the bile is limited to the intestinal canal; it does not extend beyond the mucosa. In other terms, its action is local.

The paratyphoid B culture with which we made the greatest number of our experiments, was isolated at Verdun in 1915, during a large epidemic. It came from a patient ill with a serious attack of paratyphoid fever. In 1917 we noticed that our culture began to lose its virulence, so that during the following year, we found that we could not produce a fatal infection in rabbits, after its ingestion. This setback held up our experiments for the time, but it gave us the opportunity to study the effect of intravenous inoculations.

We have already referred to the similarity of the lesions found in the rabbit after intravenous inoculation, and of those found in the chimpanzee, after the ingestion of the virus. We could then in case of necessity employ rabbits. But there were certain difficulties in the vaccination experiments in rabbits, for it was necessary to inoculate rather large doses of virus. In order to produce a fatal infection in the rabbit, after intravenous inoculation, it was necessary to inoculate as much as $\frac{1}{8}$ of an agar culture. This represents a considerable quantity of bacterial bodies.

The death of the animal in this case, is due just as much to the infection as to the intoxication by the endotoxin. We know that it is in reality a simple matter to react against the infectious element, while it is extremely difficult to neutralize the effect of the endotoxin.[1]

Two methods were open to us of overcoming the difficulty of giving such large doses—either to increase the virulence of the bacterium or to lower the resistance of of the animal.

[1] Bull. Instit. Pasteur, Vol. XII, February 28 and March 15, 1914. Annales Instit. Pasteur, July, 1905; February, 1906.

The attempts to increase the virulence of the paratyphoid bacillus did not succeed. After about 15 passages from rabbit to rabbit, the virulence of the culture was about doubled. It was necessary to repeat this animal passage quite often, in order to prevent the culture from returning to its original infective titre.

We then tried to influence the animal itself by reducing its natural resistance. We found this possible by employing bile.

Our studies have shown that when fasting rabbits are given bile by mouth, a diminished resistance is created in regard to the subsequent administration of the paratyphoid virus by mouth. It is also interesting to note that this is likewise true, when the virus is inoculated into the vein.

The following is the technic that we now employ for sensitizing the animal. The day before the inoculation of the virus, at about 5 p.m. the animal ingests 10 cc. of a mixture of bile and powdered licorice. No food is given. The next morning at 10 a.m. another dose of 10 cc. of bile is given, and 2 hours later, the virus is inoculated intravenously. The animal is then fed. Powdered licorice is given in order to mask the taste of the bile and to decrease its fluidity. This decreases the possibility of the mixture getting into the lungs.

As a result of our experiments it is shown, that rabbits prepared with bile, die after the intravenous inoculation of a much smaller dose of virus, than non-prepared rabbits of the same weight. The same result follows in rabbits infected by the mouth.

No matter what the portal of entry of the virus may be, the previous ingestion of bile, tends to diminish the natural immunity of the animal. The same action produced by bile, following ingestion or intravenous inocu-

lation of virus, is another proof, that the intestinal wall is the receptive organ.

We can easily understand the mechanism of the decrease of immunity that follows, after the administration of bile. Bile being a powerful cholagogue, has a desquamating action on the superficial layer of the intestine. This layer being removed, the remains of the mucus are swept before it, and so the intestinal wall becomes particularly permeable. A break is therefore made in the intestinal wall, and because of this, the paratyphoid infection becomes established.

In the rabbit not sensitized by bile, the resistance to the virus is assured by the integrity of the intestinal wall. The natural antiparatyphoid immunity is in a large measure local.

To summarize; the rabbit is an animal which is normally slightly susceptible to the paratyphoid virus, but becomes so, after the ingestion of bile. Considering the mode of action of the bile, the cause of the considerable resistance in this animal to the paratyphoid bacillus, rests in the intestinal wall.

The part played by bile in typhoid infections in guinea-pigs was recently demonstrated by Sedan and Herrmann.[2] These workers employed a particularly virulent strain of Eberth bacilli—a strain which had been obtained from the blood of a patient suffering from typhoid fever. Guinea-pigs that had fasted for 2 to 3 days, were injected with increasing doses of bacteria in the muscles of the lumbar region.

At autopsy profuse intestinal lesions were noted—diarrhea with Eberth bacilli in the feces, in the liver, in the spleen and in the intestine. Blood culture was

[2] C. R. Soc. Biol., Vol. XC, February 26, 1924, p. 567.

negative. In order to obtain these results, large quantities of bacteria were injected. The author remarked, "we have verified the necessity, in reproducing Besredka's experiments, of inoculating a much smaller dose of virus, in order to produce a general infection, provided 2 ingestions of 10 cc. each if bile are given a day or two before."

These workers regard the action of bile in fasting guinea-pigs, to be due to an epithelial desquamation—quite like that observed in our rabbits that were sensitized with ox bile.

Sedan and Herrmann have also succeeded in producing characteristic intestinal lesions in guinea-pigs, after sensitization with bile, and subsequent subconjunctival inoculation of typhoid bacilli.

No matter then what the portal of entry of the virus may be—whether oral, intramuscular, intravenous or subconjunctival—the organ that enters in reaction with it, is always the same. The virus invariably travels toward the intestine.

We may then repeat here, what has been said in the previous chapter regarding the mechanism of dysentery infection. The dose of paratyphoid bacteria that infects, is the one that comes in direct contact with the intestinal wall. In order to obtain the same pathological effect, the initial dose of virus must vary, depending upon the portal of its entry, because the loss of virus varies between the point of inoculation and its arrival at the susceptible organ. This is also why the dose must be massive after intramuscular injection, less so after intravenous injection, and still less, after sensitization with bile.

Before completing the chapter on the mechanism of typhoid and paratyphoid infection, it may be of interest

to record two facts, which may throw light on the part played by bile in the intestinal tract. One pertains to alimentary anaphylaxis and the other to the absorption of tetanus toxin from the intestinal tract.

Makaroff[3] began with the hypothesis, that hypersensitiveness to alimentary products taken by the mouth, depends upon the permeability of the intestinal canal. This idea appeared quite possible, as alimentary anaphylaxis could be obtained in young animals, but was almost impossible in adult animals.

The writer employed bile, in order to verify the hypothesis of the relationship of the permeability of the intestine in anaphylaxis. One series of adult guinea-pigs were sensitized *per os* with milk and no bile, while another series were given milk and bile. Only the guinea-pigs of the second series appeared hypersensitive when subsequently tested. It was shown then that guinea-pigs, even though adult, reacted with an anaphylactic shock after oral administration, and that the key to the phenomenon was due to the permeability of the intestinal wall.

Indirectly, it was shown that bile has the ability to render the intestine permeable to a high degree.

An experiment of a similar nature was made by Dietrich. This worker fed tetanus toxin to white mice. Each mouse was fed 0.4 cc. of ox bile with a tube, and 2 hours later they were given 0.4 cc. of tetanus toxin (4,000 fatal doses). The mice treated in this manner died of tetanus. Those that received toxin only (0.4 cc.) *per os* without bile survived.

The writer concludes that after the ingestion of bile, the intestine acquires the property of absorbing enor-

[3] C. R. Soc. Biol , Vol. I, June 3, 1922, p 1160.

mous doses of tetanus toxin, while there is practically no absorption in non-prepared animals.[4]

The permeability of the intestinal wall after ingesting bile, which was studied in regard to the paratyphoid virus, finds experimental confirmation for substances which are quite foreign, such as milk or tetanus toxin.

From the point of view of the mechanism of its infection, paratyphoid infection may be regarded, in a large measure, as an entero-infection. This point being established, we come to the problem of the mechanism of antiparatyphoid immunization.

II

The war, with its consequent number of typhoid cases, has given us a valuable idea of the efficacy of the usual antityphoid vaccines. No one doubts that they rendered valuable service, but it also cannot be denied that the vaccine does not absolutely prevent the infection from appearing in vaccinated individuals. We will return to this later.

We will examine the problem of vaccination before the war, from an experimental point of view. We had a number of vaccines—all of which, in the minds of their originators, were capable of producing immunity against the typhoid fevers. In order to confirm their opinion they depended on the guinea-pig. A vaccine was re-

[4] Klin. Wochenschr., Vol. I, June 3, 1922, p. 1160.

Before reporting his experiments on tetanus toxin, Dietrich summarized our work on dysentery and typhoid and paratyphoid infections He stated that he was able to confirm them, but added that he did not agree with our conception of local immunity, for, during the course of his experiments, he was able to demonstrate agglutinins in the serum of animals that had previously absorbed typhoid and paratyphoid bacilli Dietrich believed there is a pure humoral immunity following vaccination *per os*, and that bile only facilitates the absorption of the antigen by the intestinal wall.

garded efficacious, when it was able to protect a guinea-pig against a typhoid peritonitis.

When we recognized the real experimental typhoid fever, such as occurs in the anthropoid apes, the problem of antityphoid vaccination entered a new phase. Bacteriologists lost confidence in the guinea-pig as an experimental animal.

Metchnikoff and we also undertook a series of experiments on chimpanzees and gibbons. Amongst our various purposes, we wished to control the efficacy of different vaccines. We started by studying the vaccines most generally employed at the time, namely, dead cultures and autolysates employed by Vincent.

We will not enter into the details of those experiments. We will merely state that they were not favorable for these preparations. The vaccinated chimpanzees, when subsequently tested by oral infection, contracted typhoid fever in the same manner as the non-vaccinated controls.

We further made a number of other experiments. Amongst these, two in particular may be recalled here. This is a brief summary:

The first experiment was made on two chimpanzees. One ingested 15 cc. of a heated typhoid culture (60°), taken at two intervals. The other chimpanzee was employed as a normal control. Eleven days later each of the two animals received by mouth, ½ of an agar culture in a Roux flask, of living typhoid bacilli.

The chimpanzee that had received the heated bacteria by mouth, showed a slight elevation of temperature. At three different intervals blood cultures were made, and each time they remained sterile.

The control chimpanzee, infected in the same manner as the preceding animal, developed a sharp rise of temperature, which lasted several days. Two blood cultures,

taken at different times, gave a positive result. Even when the temperature began to drop, a blood culture proved positive.[5]

This experiment appeared quite conclusive. We felt so at the time. But we thought it would be advisable to repeat it. We decided to make the test rigid, so as to have all the chances against us.

On July 22, 1910, we gave a chimpanzee by mouth, 3 cc. of a heated typhoid culture (instead of 15 cc.), and repeated the same dose two days later. Ten days after the beginning of the experiment, we made our test inoculation. In order to make our proof demonstrative, we employed viruses that had come from several different sources. We had our chimpanzee swallow cultures coming from two fatal cases of typhoid fever, with cultures originating from three typhoid anthropoid apes, and then added fresh fecal material, obtained from a serious case of typhoid fever at the Pasteur hospital.

Six days after the bacterial meal, which was large and very virulent, the animal developed fever, which lasted several days, and a positive blood culture.

These results were disconcerting. As we had succeeded so well in the first experiment, and as we were certain to be able to vaccinate by the oral route, we did not hesitate to employ a very large dose of virus in the second experiment. This proved to be an error. We realized this in drawing our conclusions and thought it advisable to be cautious. "New experiments should be made; only in vaccinating man, it is necessary that they be done under more precise conditions, than those which have been made up to the present."[6]

Since we were unable to purchase any more anthropoid apes, we were compelled to give up these experiments.

[5] Annales Institut Pasteur, March, 1911, p. 210.

[6] Loco citato.

After these experiments on the chimpanzee, two publications appeared in France, on vaccination by the mouth. One in 1911, by J. Courmont and Rochaix, the other in 1914, by A. Lumiere and Chevrotier.

The experiments of J. Courmont and Rochaix were made on the goat, guinea-pig, rabbit and man. The method of introduction of the virus was either by the mouth, or directly into the intestine by way of the rectum. According to these workers, immunization was obtained by the two methods. They preferred introducing the vaccine by rectum, because following ingestion there was occasional malaise, and because the vaccine was poorly tolerated, appeared less efficacious. Each rectal dose was 100 cc. for the rabbit, and from 250 to 300 cc. for the goat. Three rectal doses were given at intervals of several days.

After a few days, agglutinins, bacteriolytic and bacteriocidal substances appeared in the serum. After 10 to 15 days, the animal was protected. The rabbits that had received three rectal doses of 100 cc. each, resisted an intravenous inoculation of 1 cc. of a typhoid culture. This was sufficient to kill the control animal in 28 hours.[7]

Starting with the idea that, "the attempts of Metchnikoff and Besredka seemed to demonstrate, that bacterial products may be absorbed by the intestinal canal, and are susceptible of producing a certain degree of immunity," A. Lumiere and Chevrotier carried on vaccination experiments on guinea-pigs and rabbits.

"With a dose of 3 billions of bacteria for each kilo of body weight—ingested in 3 doses, at 8-day intervals—we were able to obtain a definite durable immunity in

[7] C. R. Acad. Sciences, March 20, 1911, p. 797; April 10, p 1027.

guinea-pigs and rabbits, against an experimental typhoid paratyphoid and colon bacillus septicemia.

"Four months after the vaccination, the immunized animals could resist an inoculation of a fatal dose of the corresponding culture, without presenting the least disturbance. The controls, inoculated in the same manner, died in 24 hours."[8]

In Germany, between 1906 and 1910, a number of publications appeared on paratyphoid vaccination by the mouth.

Loeffler in 1906 observed that on feeding mice with dead cultures of mouse typhoid bacilli, they would be protected against a fatal infection. Mice could never be vaccinated by injections made under the skin or by intraperitoneal injections.[9]

The same year, Kutscher and Meinicke observed that after guinea-pigs had ingested paratyphoid cultures, they were able to resist, four weeks later, 1,000 to 10,000 times the fatal dose, injected intraperitoneally.[10]

Wolf in 1908 obtained the same results as Loeffler, after vaccinating mice *per os* with living, but completely avirulent cultures.[11]

Bruckner in 1910 succeeded in immunizing mice against a subcutaneous inoculation of paratyphoid bacilli by previously mixing living bacteria in the food.[12]

It appears from all of these experiments, that vaccination by the mouth against the bacteria of the typhoid group is not only possible, but readily effected. It is interesting to note, that all the authors, except Loeffler,

[8] C. R. Acad. Sciences, January 19, 1914, p. 197.
[9] Leuthold's Festschrift, 1906, p. 243.
[10] Zeitschr. f. Hyg., Vol. LII, 1906, p 370.
[11] Munchener mediz. Woch., 1908, p 270
[12] Zeitschr. f. Immunitätsf., Vol VIII, 1910, p. 439.

thought that the immunity obtained, was a general one. They based their opinion on the fact that the serum of the vaccinated animals contained agglutinins and other antibodies. They particularly thought that the immunity was general because animals vaccinated by the mouth, were able to resist, not only the ingestion of the virus, but also its subcutaneous inoculation. These hypotheses were of importance at the time; but we know their value to-day. This was the state of the question of vaccination *per os*, before the war.

We had an unusual field for observation during two years of the war (1914–1915 and 1916–1917) in sectors that were heavily infected with typhoid and paratyphoid fevers. Amongst the thousands of cases of typhoid fever that came every day from the Argonne and the Woevre, we found that a number of patients had never been vaccinated, while others had not been vaccinated for some time or incompletely so. There were others however, that had been vaccinated in the prescribed manner during the year preceding their admission to the hospital. Amongst these, there were many seriously sick cases. Many of them died of typhoid fever.

It should be noted, that in our survey, we called a case typhoid fever only when the blood culture was positive.

From these observations, which were made during the first year of the war, in hospitals at Verdun, and the next year at Bar-le-Duc, it was quite evident that the ordinary method of vaccination, by subcutaneous injection, was undoubtedly useful; but it was also quite clear, that it did not absolutely protect one from contracting typhoid fever. It was quite clear that the immunity conferred by vaccination was not as solid as that obtained after an attack of the disease.

We were impressed with these observations, and decided to study the problem of typhoid vaccination on our return to the laboratory. We desired to study the problem from a purely experimental point of view, especially in an attempt to elucidate the mechanism of vaccination and the nature of the immunity acquired.

It was quite evident to us that laboratory animals could be immunized by the oral route. Metchnikoff's and our experiments on the chimpanzee, and those of many other experimentors on mice, left no doubt on the subject. The point in which we were particularly interested was the explanation of the intimate process of immunization following the oral route.[13]

The first point to be decided upon was the choice of animal for our experiments. It was impossible to think

[13] We wish to recall our experience with the mouse, made before the war, in collaboration with Miss Basseches (Ann. Instit. Pasteur, Vol. XXXII, p 193). During the course of our experiments, we noted the following One day, having no mice for our control experiments, we decided to employ a mouse that had received, about a month previously, some paratyphoid bacilli by the mouth. The next day we were surprised to note that this animal, which had been injected the day before with $\frac{1}{100}$ of a virulent paratyphoid culture—a certain fatal dose—was eating its food exactly as if nothing had happened. Thinking that we had made a mistake, we tried other mice of the same batch. They also survived. With these unexpected results in mind, we undertook a series of experiments, employing hundreds of mice, and varying the nature of our experiments. We were forced to accept the fact that the virus introduced *per os* conferred an immunity—an idea that up to that moment was regarded as being the property of the virus, when introduced under the skin. Believing that the mechanism of immunity must be the same in both instances, we concluded that the intestine must play an active part when the animals are vaccinated by the subcutaneous route. If this were true, then the virus inoculated under the skin, must pass the intestinal wall at a certain time and appear in the lumen of the bowel. The war interfered with our experiments for four years. Just as soon as we returned to our laboratory, we again took up these experiments on rabbits. These results were the basis of our first publication after the war. (C. R Acad Sciences, Vol. CLXVII, p. 212, July 29, 1918.)

of employing chimpanzees. Could we employ mice? Their size was not compatable with the nature of the experiments we had in mind. The study of the mechanism of immunity required frequent bleedings in order to make serum studies. The guinea-pig and rabbit remained. We decided to employ the latter, as we already knew its reaction to typhoid and paratyphoid infections, and especially the precise mechanism of that infection (see above).

In choosing the rabbit we ignored an important detail, which rendered our earlier experiments difficult of interpretation. We did not expect to find it so difficult to vaccinate this animal. Until then, all the animals employed, such as anthropoid apes and mice, developed a solid immunity following the ingestion of virus, even when the virus was killed. But the rabbit could swallow living or dead paratyphoid bacilli without showing the least trace of immunity later. It was a great surprise to us, to see living and virulent cultures of paratyphoid bacilli pass the intestinal canal of the rabbit without leaving the least trace of the passage, and without being followed by the slightest degree of immunity.

We thought that there must be some interference between the virus and the intestinal wall of the rabbit, thus making this animal react differently from other animals.

Because of our previous experiments on the mechanism of paratyphoid infection in the rabbit, we knew what effect the ingestion of bile would have. The later experiments were thus indicated. It was only necessary to facilitate the contact between the ingested virus and the intestinal wall. This was accomplished by giving bile, which removed the superficial epithelial layer of the intestinal wall.

We quote an illustrative experiment:

Experiment. Rabbit A received *per os* 10 cc. of bile mixed with licorice powder (July 23, 1919) at 5 p.m. Animal remained fasting The next day at 10 a.m. animal was fed with 10 cc. of bile, mixed with licorice. At noon (July 24), ½ of a Roux flask of living paratyphoid bacilli was given by mouth. Animal was then fed as usual.

Seven days later (July 31) the rabbit weighed 1,670 grams. Even though the animal had not completely returned to its normal state (it had lost 200 grams since July 23) the test inoculation was made Following the technic adopted, the rabbit was first sensitized (July 31 and August 1) and then inoculated intravenously (August 1) with $\frac{1}{20}$ of an agar culture of paratyphoid bacilli.

A control Rabbit B, 1,830 grams, sensitized at the same time as the former (July 31 and August 1), was inoculated (August 1) intravenously in the same manner ($\frac{1}{20}$ of a culture).

Rabbit A, lost as much as 300 grams directly after the inoculation. It subsequently regained its weight and survived.

The control Rabbit B developed diarrhea the day following the inoculation. It died during the night of August 5. At autopsy (1,380 grams), the characteristic lesions were found. Since these lesions have already been described, repetition is unnecessary.

This experiment demonstrates that a rabbit, previously prepared with bile, and then having ingested the living paratyphoid virus, acquires an immunity. This immunity is definite, for in spite of the fact that the test inoculation of a fatal dose is given intravenously, the animal resists the injection and survives.

The immunity acquired only in the sensitized rabbit—that is, in an animal that had a slight paratyphoid infection—leads us to believe that it is the same kind of immunity as is acquired in man following a typhoid or paratyphoid infection.

This analogy opens the possibility of studying the mechanism of typhoid and paratyphoid immunity in man.

As instructive as this experiment may be, it is mainly

of theoretical interest. No one would suggest the ingesting of living bacteria as a means of vaccination. Still this experiment is very instructive, for it shows that the previous ingestion of bile completely changes the possibility of absorption of the virus. We may now ask, whether a certain degree of immunity may not be obtained in the rabbit, by giving bile and then having it ingest dead paratyphoid bacilli. The results would be of great interest, even though the immunity acquired might not be as solid as that obtained after the oral administration of living virus.

This is one of the experiments:

RABBIT A	RABBIT B	RABBIT C
February 6, 1919. 2,070 grams. Received *by mouth, after preparation with bile*, ½ of an agar culture in a Roux flask, of paratyphoid B—heated (60° for one hour)	February 6. 2,150 grams. Received *by mouth, without previous preparation*, ½ of an agar culture in a Roux flask of paratyphoid B—heated (60° for 1 hour)	February 5, 6. Sensitized with bile and no culture
February 27. 2,420 grams. Inoculated intravenously, after sensitization by bile, with ½ of an agar culture of paratyphoid B	February 27. 2,520 grams. Inoculated intravenously after bile sensitization, with ½ of an agar culture of paratyphoid B	February 27. 2,350 grams. Inoculated intravenously, after bile sensitization with ½ of an agar culture of paratyphoid B
Survived	March 2. Dead of typical paratyphoid infection	March 3. Dead of typical paratyphoid infection

A priori, the probable success of such an experiment was not expected. Since the rabbit could not be vaccinated after the ingestion of living virus, we could hardly expect to vaccinate with dead bacteria. The

only possible hope was, that this experiment might succeed in animals sensitized with bile—that is, in an animal where the intestinal wall was more permeable than ordinarily.

To summarize, the two rabbits (A and B) had ingested, under identical conditions, heat-killed cultures of paratyphoid bacilli. Rabbit A was previously sensitized with bile. Three weeks later the two rabbits (A and B) with a control (C) were submitted to a test inoculation by intravenous injection of a fatal dose of virus, after a prior sensitization.[14]

Control (C) died of a paratyphoid infection. Rabbit (B), the animal that had absorbed dead bacteria alone without bile, also died. The only animal to survive was rabbit (A), or the one that had been given bile before it absorbed heat-killed bacteria.

The results of the experiment show that the permeability of the intestinal wall, following the ingestion of bile, permits the absorption of the heated bacteria and so establishes an immunity against living virus.

We then have a method of vaccination, which is harmless, and which imitates to a certain degree, the conditions as they occur in nature—that is, the immunity which follows a mouth infection of typhoid or paratyphoid fever.

This vaccination *per os* is rapid in the rabbit. As a result of our experiments we find that the animals

[14] The previous sensitization with bile before making the test inoculation is important. We obtain a fatal infection with a relatively small dose of virus. Some authors, that attempted to repeat our experiments, failed to carry them out in this manner.

During the course of each attempt at vaccination there are two sensitizations to be practised. The first, to render the intestine permeable to the vaccine, and the second to render the intestine permeable to the virus.

acquire an immunity as soon as three days after vaccination. There are three advantages to the use of the oral vaccine—innocuousness, effectiveness and rapidity of action.

What is the mechanism of the immunity acquired by this procedure? For a long time we believed, with most bacteriologists, that the immunity following the ingestion of antigens, as well as that following their injection elsewhere in the body, depend upon the presence of antibodies. We were particularly led to this idea, because in our animals vaccinated *per os*, we always found antibodies.

Gradually our faith in the effectiveness of antibodies lessened. The amount of antibodies in the serum does not always correspond to the degree of immunity. For, if we feed rabbits with paratyphoid bacilli, without giving them bile, we often find the presence of antibodies, and still these animals show no immunity. On the other hand, we have often been able to immunize rabbits solidly with dead cultures and bile, and have never been able to demonstrate antibodies. We were also surprised to observe the reverse. One day, while desiring to increase the immunity in rabbits, by repeated ingestions of bacteria, we found that the agglutinins, which appeared at the beginning of the vaccination, gradually diminished after the second bacterial meal. They finally completely disappeared after many ingestions of the antigen.

We therefore sought to explain the cause of the immunity by conditions other than by the presence of antibodies. We wondered whether the immunity did not reside in the intestine, perhaps in the follicular cells. The point in favor of this view was the fact that bile was so important, in fact necessary, in establishing the immunity.

What does bile do? As we have already stated in regard to the mechanism of infection, it denudes the mucous layer of the intestine and facilitates a direct contact between the receptive cells hidden below the mucosa, and the bacterial bodies contained in the vaccine. The receptive cells are then impregnated with the specific antigen and so lead to the immunity of the animal.

On reflection, we find that the mechanism of the immunization, is in reality the same as the mechanism of infection. The two processes only differ in their intensity. The receptive intestinal cells, on entering into reaction with the living paratyphoid antigen, undergo a violent reaction, causing infection and death. When the reaction between the receptive cell and antigen is feeble, as occurs after the ingestion of dead virus, we have the production of an immunity.

The rabbit, not sensitized with bile, is naturally refractory to the ingestion of virus. This is due to the fact that the mucosa acts as a barrier between the virus and the receptive cells. This is why the virus may pass the intestinal canal without leaving any trace of its passage.

On the other hand, the rabbit that has been sensitized with bile has its receptive cells uncovered. The paratyphoid antigen on passing through the intestine is seized by the receptive cells with which it enters in action. At a certain moment this affinity of the receptive cells becomes satisfied. When, sometime later, a new infection occurs, the new virus finds itself in contact with intestinal cells that have lost all their affinity for it. The cells, having become non-receptive, are unable to react; and the virus, having become inoffensive, produces no infection. In other words, the same phenom-

enon takes place as that which has been described in anthrax infection. No matter how virulent a bacterium may be for a new animal, it ceases to be dangerous in the vaccinated animal, because it does not find receptive cells.

Natural immunity in the rabbit, to the paratyphoid virus, depends upon the barrier which the intestinal mucosa opposes to its penetration. When we are dealing with artificial immunity this barrier becomes real. In order to break it down we have employed the particular properties of bile.

We do not say that the same end may not be achieved by other means. In dysentery, for instance, bile is not necessary, for the dysentery bacilli may denude the epithelial cells by themselves. In certain species of animals, that are particularly receptive to the typhoid and paratyphoid virus, the use of bile may be omitted. We refer to our experiments on the chimpanzee and mice. Both of these animals may be vaccinated by the oral route, without employing bile. We believe that even in these animals the use of bile may be helpful, for it permits the virus-vaccine to give its maximum effect. The gastrointestinal medium in which we operate, is susceptible to many changes. The state of its fullness changes from moment to moment; the presence of ferments and the reaction of its contents may also vary. By preceding the ingestion of the vaccine by ox bile, we eliminate, as far as possible, various causes which may render the vaccine ineffective. These are the reasons, we believe, why the previous administration of bile may be useful in man, even though he is particularly sensitive to the typhoid and paratyphoid virus. Bile, aided by the favorable action of fasting, and because of its varied

action already described, assures the maximum effect in producing the intestinal immunity.

Vaccination by means of heated cultures and bile, has already been employed in man. About 200,000 people have selected the oral method rather than subcutaneous inoculation. This method of vaccination has been employed during many epidemics, and the results have served as a basis for many communications.

In order that this chapter should not be too long, we will only report the results of a typhoid epidemic at La Fleche in 1923, and another in Roumania in 1924.

A particularly serious and fatal epidemic broke out at the military school at La Fleche (Sarthe). The first case was detected on April 8, 1922. On April 18, there had occurred, among the students in the school, 29 cases and two deaths. Besides this group, 12 other cases occurred among students who were home on their vacation. In addition, the janitor and a keeper came down with the disease—making in all 43 cases in a period of ten days.

A general of the medical corps immediately ordered vaccination for all the students. A large number of students could not be vaccinated in the ordinary way, because of their physical condition, or because some had organic disease. More than one-half of the students, or 268, belonged to this class. By order of the General, all these pupils were vaccinated by the mouth, with the bile vaccine.

All the other healthy students—253 in number—were vaccinated with the T.A.B. vaccine[15] by subcutaneous injection. The subcutaneous injections were made on April 19 and 20. The ingestion of the bile vaccine was made on April 22, 23 and 24.

[15] Typhoid-Paratyphoid A and B vaccine, heat-killed.

As was expected, the epidemic did not stop immediately. Shortly after the vaccination, new cases occurred in individuals who had been vaccinated during the period of incubation.

Among the group treated with the bile vaccine (268), five new cases occurred, between April 29 and May 5, or during 11 days directly after the treatment. Among the group vaccinated by subcutaneous injection (253), 10 new cases occurred between April 27 and May 10, or 20 days following the vaccination.

No deaths occurred among the vaccinated individuals. In them the disease was mild. Vaccination by the mouth produced no disorders and was readily taken by all.

As has been indicated, the epidemic was severe and the authorities deemed it advisable to employ the two forms of vaccination. The number of persons vaccinated by the subcutaneous method was about equal to those who were vaccinated by mouth.

This study, which is in the nature of a laboratory experiment, may be summarized as follows. A group of students, all living under the same conditions, were overcome by an epidemic of typhoid fever. Forty-three came down with the disease. Directly thereafter 253 of the healthiest individuals received the T.A.B. vaccine subcutaneously, and 268 of the weaker students received the bile vaccine by mouth.

At the end of the epidemic it was found that, of those vaccinated subcutaneously, 10 contracted the disease during the following 20 days. Of those vaccinated by the mouth, 5 contracted the disease during the 11 days following the vaccination.

Should we conclude that the bile vaccine is twice as efficacious as the T.A.B. vaccine, or that the immunity acquired after the ingestion of the vaccine is established

twice as fast as that by injection? We would be tempted to do so, especially if we refer to our rabbit experiments, in which the rapidity of the immunization following ingestion was demonstrated. While awaiting further statistics, we shall only draw the following conclusions from the experiment at La Fleche.

1. The ingestion of the typhoid bile vaccine is inoffensive, even in subjects where the subcutaneous vaccination is contraindicated.

2. Vaccination *per os*, with bile vaccine, is at least as efficacious as subcutaneous vaccination with the T.A.B. vaccine.

This is the report of the epidemic at Moreni. While it is not as demonstrative as that at La Fleche, still it is instructive.

Moreni is one of the principal oil centers in Roumania. From September 1923, to July 1924, there were 54 cases of typhoid fever, and 9 deaths. These figures are probably underestimated as Cantacuzene and Panaitescu, to whom we owe the report of this epidemic believe. At the time that these investigators carried on the vaccinations (July, 1924) Moreni had a constant population of 4,800 and a moving population of 11,734; in all 16,534 individuals. These were divided in three groups.

8,673 were vaccinated by the subcutaneous method (T.A.B. heated 56° for one hour).

2,286 were vaccinated by mouth (bile vaccine).

5,575 acted as controls.

Of the first group of 8,673 individuals 1,434 received only one injection. 2,693 received two and 4,546 received the three. Of 2,286 individuals, who received the bile vaccine, 314 ingested the vaccine once, 372 twice, and 1,600 three times. Cantacuzene and Panai-

tescu stated, that in spite of their recommendation, some people took the bile vaccine after having had their breakfast.

These were the results on January 17, 1925, or 6 months after the vaccinations.

Of 5,575 non-vaccinated, 90 cases of typhoid fever, 6 deaths.

Of 2,286 vaccinated *per os*, 6 cases of typhoid fever, no deaths.

Of 8,673 vaccinated by subcutaneous injection, 3 cases of typhoid fever, and 3 deaths.

The authors state that these figures are open to criticism because it only considers the individual who remained in the city; for a number of people vaccinated and non-vaccinated left the city during the course of the experiment.

Another cause of error, which is very important, is the fact that we do not know the number of individuals who had eaten before taking the bile vaccine.

With these two reservations, the results in the vaccinated and non-vaccinated are still of enough importance to conclude with Cantacuzene and Panaitescu, that "the efficacy of the two methods is definite."[16]

We wish to note some objections which have been raised regarding the use of bile. They refer less to the use of bile, than to the dose employed.

A long series of experiments would be necessary to determine with precision the exact number of cubic centimeters of ox bile that are necessary to sensitize the intestine of the rabbit or of man. In our experiments, we employ 10 cc. of bile. Perhaps a much smaller dose would have been sufficient for the rabbit. We do not

[16] C. R. Soc. Biol., Vol. XCII, May 1, 1925, p. 1138.

know. The question is of little importance, considering the ease with which bile may be obtained, and the facility with which the rabbit takes it.

It has been remarked, that if the rabbit needs 10 cc. of bile, for example, we should employ 300 cc. to sensitize the intestine of man. Admitting that the figure for the rabbit can not be reduced, we feel that a parallel calculation cannot hold true. For we do not take into consideration the sensitivity of the species and only consider the weight of the animal. The discussion is more mathematical than biological.

It has also been said that if we take into consideration the quantity of bile excreted by man daily, the small amount of ox bile which is given before the vaccine, can have no effect.

If this were true, bile would never be employed in human therapeutics. As a matter of fact bile has been employed since 1300 B.C., as is indicated in a parchment of that period. Gallen employed bile and called it the natural enema; Louis XIV was a warm partisan of it.

In more modern times Roger (1924) also called attention to the favorable use of bile in various intestinal infections. "We advise an extract of ox bile in cases of muco-membranous enteritis. With this treatment the false membranes disappear and the pain, which accompanies their expulsion, also diminishes." "The use of ox bile," he adds, "also has the advantage of activating the liver function and counteracts the constipation in the patient."[17]

Bile is also regarded as effective in hepato-biliary affections, such as lithiasis, cholemia, angiocholitis and cholecystitis. Man may then secrete large quantities

[17] Questions actuelles de biologie medicale, Masson, 1924.

of bile, but he may still derive some benefit from an additional small quantity.

Another objection raised by ourselves, may be more serious than those of our critics. Ever since the mouth vaccine has been applied to man we wondered whether we had not surpassed our experimental rights, in recommending a method to replace the subcutaneous method—which is now supported by more than 30 years of experience. In the beginning, our conscience bothered us greatly. Now that favorable reports of its use in epidemics are being made from various sources, we are not so troubled. We still hold a certain reserve, for if the principle of oral vaccination is correct its practical application may need some perfection.

If we consider that the ordinary method of vaccination by subcutaneous injection is not perfect, our responsibility is not so great. We all know of its inconveniences, which appear to be of little importance. But we know of definite instances where the injected vaccines produced serious results and did not give the desired effect.

Many cases of this nature were observed during the war. Among the various authors who called attention to it, may be mentioned Pierre Hébert and Marcel Bloch, who studied a great many cases. Between July 1916, and January 1919, these authors made systematic blood cultures on all cases of fever that were admitted to the military hospital at Bar-le-Duc. They obtained 2,334 positive blood cultures, divided as follows:

431—Eberth bacilli
1,598—Paratyphoid A
305—Paratyphoid B

Among these 2,334 cases of typhoid infections, there were:

1,249—non-vaccinated (or 53.5 per cent)
616—incompletely vaccinated (26.4 per cent)
469—*completely vaccinated* (20.1 per cent)

Taking into consideration the large number of patients that had been completely vaccinated (469), it was interesting to determine when the disease occurred, or more exactly, the interval between the vaccination and the infection. This study showed:

28 vaccinated individuals contracted the disease during the month that followed the vaccination
37 vaccinated had a positive blood culture after two months
16 vaccinated had a positive blood culture after three months
24 vaccinated had a positive blood culture after four months
38 vaccinated had a positive blood culture after five months
54 vaccinated had a positive blood culture after six months, etc.

It was these striking figures, obtained by careful control in the laboratory and clinic, that stimulated us to begin our prewar experiments, just as soon as we returned to civil life.

The results of our recent experiments, added to the failures of subcutaneous vaccination seen during the war, justify we feel, attempts at oral immunization in man.

CHAPTER V

Theory

I. *History.* Methods of vaccination against snake-bite, smallpox, and peripneumonia. Period of Pasteur. Vaccination against chicken cholera. Theory of exhaustion. Humoral theory of German scientists. Pfeiffer's conception. Theory of the two substances of Bordet. Cellular theory of Metchnikoff. Side-chain theory of Ehrlich.

II. *Natural immunity against anthrax in the animal kingdom.* Larva of beetle, cricket, slug and snail; fish, frog, crocodile, chicken, pigeon, dog and rat Function of chemotaxis.

Virulence—its relativity depends upon the species, and in the same species, depends upon the tissue. Anthrax in the guinea-pig.

Anthrax in the normal and vaccinated rabbit; mechanism of infection and immunization in each. Rôle of chemotaxis. Function of the receptive cells of the skin and of the white blood cells.

Receptive cells of the intestinal wall. Ingestion of bile, its importance in natural and artificial immunity. Respective function of the receptive cells of the intestine and of the white cells

III. *Accustoming cells to poisons in general* Accustoming receptive cells of the skin and intestine during immunization. Mechanism of acquired immunity, regarded from the point of view of the theory of local immunity.

Analogy with anaphylaxis. Immunization, regarded as a desensitization of the receptive cells.

Mechanism of artificial vaccination. Antivirus. Function of phagocytes. Disintegration of bacterial bodies and liberation of antivirus. Production of antibacterial cytolysins.

IV. *Function of antibodies in acquired immunity.* Agglutinins, bacteriolysins, sensitizing substance Absence of parallelism between antibodies and immunity. Conception of immunity regarded otherwise than due to antibodies. Antibodies considered as products of excretion.

Function of antibodies in passive immunity. Combined action of antibodies and antivirus. Unity of the mechanism of immunity, in spite of the apparent differences in regard to the duration of immunity and the rapidity of its production.

Vaccinotherapy—function of the receptive cells. Mechanism of vaccinotherapy; its preventative character. Function of contact in biologic phenomena. Conclusion.

I

As far back as we may look into the early history of our science, we find evidence of the idea of vaccination and immunity.

The primitive people, actuated by the instinct of self-preservation, developed ideas that would be worthy of our contemporaries. The savage Vatuas from oriental Africa showed evidence of this remarkable intuition, in treating serpent bites by making cutaneous incisions in the arms and legs, and then applying a paste, which contained the specific poison. We must also consider the Achantis as our predecessors, the Siamese, and the Chinese, who from time immemorial put specific crusts into the nose and lesions of the skin for protection against smallpox. It is also interesting to note the active technic employed by the Maures of the Senegambia, who protected their animals against peripneumonia, by plunging a spear into the lungs of an infected animal and then applying the material obtained to the skin of healthy animals. Does not the method of Willelm consist in producing an incision under the surface of the tail of a healthy animal and applying the serous liquid obtained from an animal infected with peripneumonia?

Our ancestors then possessed methods of vaccination in their prophylactic armamentarium, which were just as effective as those employed by us to-day. Hence they practised cutaneous vaccination a long time before we did.

The scientific phase of experiments on immunity began with Pasteur. He was the first to demonstrate the importance of bacteria in causing infectious disease. It was also he, who first conceived the idea of employing pathogenic bacteria to therapeutic ends.

In 1879 while he was studying chicken cholera in his laboratory, he noticed that the bacterium was very virulent for the chicken, but only slightly pathogenic for the guinea-pig.

On their return from the summer vacation, Pasteur with Roux and Chamberland, again began their studies on chicken cholera. They were surprised to observe that the culture, which had been virulent before their departure, had now become avirulent, and did not kill the inoculated chickens. The fact that struck them in particular was, that the inoculated chickens which survived, resisted an inoculation of fresh virulent virus. Two discoveries, among the most important in biology were made; namely, the attenuation of the virus, and vaccination.

The theoretical importance of this experiment on chicken cholera could not be explained. There was no instance as yet of an artificial immunity being produced with a known bacterium—a bacterium that could be easily handled and so employed for experimental studies.

Pasteur directly began to speculate on the mechanism of this immunity.

Since he was more of a chemist than a biologist, he began to look for the reason in his test-tubes. He inoculated the bacterium of chicken cholera in a tube of broth and waited 3 or 4 days. The culture was then filtered and the filtrate reinoculated with a culture of chicken cholera. The bacterium did not grow this time, and Pasteur concluded that the medium was ex-

hausted, and so became resistant to a new inoculation of bacteria.

Does not the same thing happen *in vivo*, inquired Pasteur? A normal chicken contains a great deal of a substance in its serum which feeds the bacteria of chicken cholera. A vaccinated chicken has little or none of these substances—the nutritive material having been used up by the first infection. For this reason, new bacteria may be inoculated into the chicken, but cannot survive therein. This was Pasteur's reasoning, and comprised his idea of "non-infectivity." As for natural immunity, the same principle holds true—"chickens that are cured of the disease are in the same constitutional state as animals that can not be infected with chicken cholera. These animals act as if they were vaccinated at birth, either because during their fetal development they failed to receive those elements that are necessary for the bacteria to live in, or because this nutritive material disappeared during their early life."

Whether dealing with acquired or natural immunity, he regarded the mechanism as the same. In both instances the virus does not develop because of a lack of nutritive substance. In the vaccinated chicken, the muscle has become "in some way unable to cultivate the bacterium. It appears as if during a previous culture some principle in the muscle was disposed of, and so life can not continue. The absence of this substance interferes with the development of the small organism." Pasteur concludes, "no doubt this explanation will become general, and applicable to all virulent diseases."

Pasteur's prediction was not fulfilled. This theory of exhaustion, which at first glance appeared so pleasing, had few followers in his time. We will return to this later.

The theory proclaimed by Robert Koch at the International Congress in Berlin in 1890 met with pronounced acclaim. A large group of eminent biologists, Baumgarten, Ziegler, Weigert, Fodor, Flügge, Behring, and Buchner, believed that the bactericidal power of the serum was entirely responsible for immunity. R. Pfeiffer the Scientific Director of the Robert Koch Institute, in particular upheld the humoral theory for many years. His first communication on immunity in guinea-pigs against cholera peritonitis appeared in 1894. Two years later he published under the suggestive title, "The New Fundamental Law of Immunity." In this, he described his work on the transformation of vibrions into granules and their subsequent solution and destruction in the serum of the body. The exclusive function of the serum in immunity spread rapidly to include other bacteria, particularly the typhoid bacillus.

Pfeiffer believed that the destruction of bacteria in the body, was due to a substance which circulated in an inactive state in the serum of a vaccinated animal. This substance, when injected into a new animal, was activated by the endothelial cells, and was then able to destroy the vibrions. The immunity was then, in a large measure, due to the products obtained by endothelial secretion.

The humoral theory, in complete opposition to the theory of phagocytosis, was strongly attacked by Jules Bordet, at the time a young student of Metchnikoff. While attentively studying the mechanism of the Pfeiffer reaction, Bordet noticed that the participation of the peritoneal endothelial cells was not necessary in the reaction. The young scientist had no difficulty in showing that the interesting transformation of vibrions into granules may take place *in vitro*, provided a drop of

fresh guinea-pig serum was added to the specific serum. The destruction of vibrions, that had attracted so much attention, could then be obtained by the combined action of sensitizer and alexin.

Bordet's theory of the two substances, which has greatly grown in importance, had the merit of demonstrating the respective function of the cells and the serum, in the complex process of acquired immunity. We will return to this later.

The problem of natural immunity was hardly discussed, for its mechanism appeared to all to be a function of the phagocytes.

There was a wide choice among the known infections, in order to illustrate the function of the phagocytes in immunity. Anthrax was particularly suitable for Metchnikoff's studies.

If we inject the anthrax vaccine under the skin of one ear of a normal rabbit, and under the skin of the other ear some virulent anthrax bacteria, the ear inoculated with the vaccine directly shows a circumscribed area of inflammation. A purulent exudate appears, which on examination shows the leucocytes digesting all the bacteria. The infection can not spread, as it is held up by the barrier of leucocytes. The other ear, injected with virus, presents a serous or bloody exudate, with few or no leucocytes. The bacteria are free in this liquid and grow without being interfered with. The virus meeting no obstacle, spreads and leads to the death of the animal by a generalized septicemia.

This is another example of the function of the leucocytes. If we inject each of two rabbits—one normal and the other vaccinated against anthrax, with a fatal dose of virus under the skin of the ear, the next day

the normal rabbit will show at the site of inoculation, a gelatinous edema, which is poor in white cells, but rich in free bacteria. The animal succumbs to the infection. The vaccinated animal presents at the same site, a rich exudate of white cells with phagocytosed bacteria. This animal survives. Therefore, whether we are dealing with natural or acquired immunity, the absence of phagocytosis goes hand in hand with the life of the animal. Its absence indicates rapid death.

This clinical experiment, made by Metchnikoff, is equal to the best dissertation on the function of the phagocytes. It is a summary of all immunity, considered from the standpoint of the cellular theory.

The side-chain theory for a long time attracted attention in the laboratories of the two hemispheres. It would be too long to explain it here, and furthermore, it is known by those interested in the subject of immunity.

Some thought this idea clashed with the conception of phagocytosis. They were wrong. The side-chain theory attempted to explain the intimate relations by which the bacteria joins the body cells. The theory of phagocytosis did not attempt to explain this. We cannot therefore speak of a contradiction between the theories of Metchnikoff and Ehrlich. There is to be sure, a disagreement between the two, but it is secondary; it refers to the question of alexin. According to Metchnikoff, the alexin is only free after phagocytosis, while Ehrlich believed that the alexin circulates freely in the blood. But the important point in the theory of phagocytosis however, in which immunity, whether acquired or natural is a process of digestion, is not in disagreement with the side-chain theory.

This is a rapid sketch of the principal tendency of the problem of immunity as considered at the present time.

We will soon see how we may enlarge upon this conception, by virtue of the newly acquired facts.

II

One day while speaking of the humoral theory, in regard to the immunity in the dog and chicken against anthrax infection, Metchnikoff remarked, that we regard this immunity as being due to the fact that the serum does not furnish food for the bacterium; but on the other hand we know that the bacterium grows readily and kills lower species of animals, such as the cricket; and that it grows very well on carrots, potatoes, and other vegetables. Does not our common sense react against this explanation? The real explanation may be found by a direct examination with the microscope.

Experienced in the comparative method of study Metchnikoff studied the reaction in lower animals. He began with those of simple structure.

The larvae of scarabée rhinocéros are very sensitive to the vibrion of cholera, but are refractory to the anthrax bacillus. What is the reason? This was a problem, which if solved, would throw light on the causes of immunity and susceptibility.

Metchnikoff injected the anthrax bacillus into the cavity of this larva, and carefully noticed what happened. The following day all the bacteria were found inside the leucocytes—not one single bacterium was outside the cells. The ingested bacteria were treated exactly as any other phagocytosed foreign body; they were digested, and the larva of the beetle did not die. The entire explanation of the immunity rests there; the life of the

larva was saved, because of the phagocytic power of the white cells.

The conditions are different in the cricket, which is susceptible to anthrax. When the bacteria are injected into the cricket, only a part of the bacteria are phagocytosed; but the greater number remain free. The bacteria multiply in the intercellular spaces of the spleen, which is the phagocytosing organ. The cricket is rapidly overrun with bacteria and dies of a general infection.

The slug and the snail have a great immunity against the anthrax bacillus. When the bacteria are injected into the blood of these molluscs they rapidly become the prey of the leucocytes, and then disappear from the blood. As the intracellular digestion in the invertebrates takes place slowly, there is plenty of time to follow with the microscope the changes that take place in the bacteria. Engulfed in a living state, they hold their normal aspect for 10 to 12 days or even longer. They die then and are digested, without at any time leaving the white cells. These different phases, passing from life to death, may be closely followed by subculture and inoculation.

The same process of defence is found in the inferior vertebrates, especially the fish. The hippocampe, a small ocean fish, is very sensitive to the anthrax bacillus.[1] On the other hand, fresh water fish, such as the perch, the gudgeon and the gold cyprin are naturally refractory to this bacterium.[2] While the hippocampe shows at the point of inoculation with the anthrax virus, a tumefaction which rapidly leads to a fatal generalization; fresh water fish reacts differently. Directly after the inoculation, there is an accumulation of white cells,

[1] Sabrazes and Colombat, Annales Institut Pasteur, 1894, Vol. VIII, p. 696.

[2] Mesnil, Annales Institut Pasteur, 1895, Vol. IX, p. 301.

which engulfs the bacteria. Often after six hours the phagocytosis is complete. Mesnil, who made interesting observations on this subject, noticed that the exudate removed from the peritoneal cavity several days after the inoculation, could still induce a fatal infection in the guinea-pig; the engulfed bacteria were then alive and virulent.

The immunity follows the same rule in the amphibia. The experiments of Koch on frogs are classic. This scientist injected an emulsion of anthrax infected spleen into the lymphatic sac of frogs. Having found the bacteria inside the round cells, Koch thought that their presence was due to the fact that the medium was favorable to their growth. Metchnikoff however, held a different opinion. For him, it was simply a question of the leucocytes fulfilling their phagocytic function.

This opinion was vigorously attacked by Baumgarten and Petruschky. It was subsequently shown however, that it was the phagocytes which digested the bacteria and that it was the phagocytes which prevented the spores from germinating, and hence assured the immunity of the frogs.

Instances of natural immunity to anthrax are rather common in reptiles. The crocodile may be inoculated with enormous doses of virus, without producing any other than a local lesion. If we carefully examine what takes place at the site of inoculation, we find a great number of white cells, particularly mononuclears, that are filled with bacteria.

On advancing in the animal kingdom, we come to the inferior warm blooded vertebrates. Let us recall the experiments of Pasteur and Joubert. The chicken, which is naturally refractory to anthrax, contracts the disease when its legs are immersed in cold water. This

was at first explained on the basis that the normal chicken was immune, because of its temperature (41° to 42°), which prevented the bacteria from multiplying; but as soon as the temperature was lowered, the bacteria then began to grow. The experiments conducted in Metchnikoff's laboratory showed that the phagocytes alone were the cause of the immunity; and that when they were drawn away, infection occurs.

The resistance of the pigeon to anthrax was explained as being due to the fact that the bacteria could not grow in its organs. This explanation appeared logical, but subsequently it was proved incorrect. The bacteria develop very well in the organs and in the serum of the pigeon; but this animal is able to resist infection, only because the bacteria are unable to resist the voracity of the phagocytes.

We now come to the mammals. The dog possesses the greatest immunity against anthrax. This was explained because of the bactericidal property of its blood. But the real cause, as Metchnikoff proved, is the positive chemotaxis of the phagocytes for the bacterium. The destruction of the bacteria is due to a substance which is neither in the serum nor in the plasma, but which is present in the microphages.

The rôle of chemotaxis is of importance in the rat. On the behavior of this animal a great deal was placed in the discussion of the mechanism of immunity, between Behring and Metchnikoff. The rat is refractory to anthrax. Its serum is very bactericidal. Therefore, it was concluded that the immunity in the rat depended upon the bactericidal action of the serum. Was it not Behring who stated that the key to the problem of immunity rested on the question of anthrax in this rodent?

Numerous and varied experiments were made in

different laboratories, on rats of all colors: grey, white and black. We shall not enter into all the details here, but following these experiments, Metchnikoff concluded that the leucocytes in the rat contained a thermostable ferment, which acted on the bacterium. In the animal, this ferment only acts in the interior of the phagocytes. It is therefore upon this ferment that the immunity of the rat must be explained. Still, Metchnikoff's mind was too keen to feel that this wholly explained the entire condition. Even at the risk of diminishing the importance of the function of the phagocytes, he made this reservation, that before entering into the process of digesting the bacteria, the lecucocytes must undergo a chemotactic excitation. In the reverse case, that is in the absence of chemotaxis, the leucocytes may be present, but the bacteria continue to multiply.[3]

In other words, phagocytosis of the bacteria appears as a secondary phenomenon; it appears to be subordinate to a factor which is not present in the serum.

Metchnikoff did not describe the other factor. We shall attempt to do it for him. This factor is in the affinity of the bacterium for the skin.

This idea comes directly from the opinion which we hold regarding the question of the virulence of bacteria in general, and the anthrax bacillus in particular.

We realize that the idea of virulence has nothing absolute about it. For in the chicken, the anthrax bacillus acts as a saprophyte. For the rat the bacterium has a very slight virulence. On the other hand, the guinea-pig is very sensitive to it. This variation of virulence for different species is well known.

The sharp variations of virulence, which occurred in the same animal, depending upon the contact of the bac-

[3] Immunity in infectious diseases, p. 167.

terium with one or another group of cells, surprised us. As we have already shown in the first chapter, the pathogenic property of the anthrax bacillus has a wide variation: from a marked virulence when inoculated into the skin of a guinea-pig, to an absolutely inocuous microbe, when protected from contact with the skin.

The pathogenic power of the anthrax bacillus varies, depending upon the tissue with which it comes in contact. When the bacterium finds itself in the presence of non-receptive cells, it reacts exactly as in the chicken, the crocodile, the frog, the gudgeon, the snail or the beetle—it is phagocytosed quite like an ordinary saprophyte.

The guinea-pig being a higher vertebrate, possesses a receptive apparatus which is more differentiated than that in the other animals. According to our present opinion, it appears that the cells of the reticulo-endothelial layer are the receptive tissue. It is these local, fixed phagocytes in the guinea-pig and in the higher animals in general, which react in anthrax infection and immunization.

In order to make this point clear, we shall take a concrete example. Let us take the same experiment that was previously cited in reference to the function of leucocytes in anthrax infection. In one of two rabbits we inject the first anthrax vaccine under the skin of the ear, while in the other we inject active virus in the same manner. A virulent bacterium, according to our definition, possesses a great affinity for the receptive cells. This affinity is followed by a reaction between the bacillus and the cell, and a third new substance is liberated, which is a product of secretion or disintegration of the bacteria. When this substance is in a sufficient quantity, it repels

the leucocytes, and phagocytosis not taking place, the bacteria multiply and thrive.

The conditions are different where a slightly virulent bacterium is employed, such as the first vaccine. A slightly virulent bacterium, according to our hypothesis, possesses a feeble affinity for the receptive cells. The reaction that follows their union is feeble. The product of secretion or disintegration just mentioned appears in a very small quantity. The leucocytes then are not repelled, and the phagocytes may act without any interference.

Let us now see what happens in the instance of the vaccinated rabbit. What happens when we inject active virus under the skin of the ear?

We have said that a vaccinated animal is one in which the receptive cells are accustomed to the virus. Because of this, these cells have lost their affinity for the virus; they are then unable to enter into reaction with the microörganism. There is then no liberation of the substance which is capable of repelling the leucocytes. The phagocytic acitivity not being interfered with, the leucocytes are permitted to attack the bacteria, without consideration of the species, such as the anthrax bacillus, Bacillus subtilis or any other saprophyte. It should be noted that the virulence of a bacterium is not a property which is appreciated by the leucocytes. With the exception of the skin cells, no cell in the body—whether it be white cells, red cells or others—reacts in a different manner with a virulent or avirulent bacterium.

We may recall that Metchnikoff had already shown that the leucocytes are capable of engulfing bacteria in a living and virulent state. As this fact appeared to be improbable to his contemporaries, he had to devise most ingenious experiments in order to convince them. He

had to fish isolated leucocytes from the exudate that had engulfed the bacteria, and then show in hanging drop preparations, that the bacteria were really living, by watching their multiplication.

If, while inoculating the virus intraperitoneally, we are cautious not to infect the skin, we may see without any difficulty, the engulfing of the living and virulent bacteria. The leucocytes digest the bacteria easily, because the bacteria are not virulent for them. The phagocytosis in this instance, is simply due to that function of the white cells which eliminates all substances that are foreign to the body.

We see that in reality, there is only one form of chemotaxis that may be provoked artificially; namely, negative chemotaxis. As for positive chemotaxis, it need not be provoked, as it is an inherent property of the white cells. In other words, no matter what the foreign body may be—whether it be a mineral or organic substance, a saprophytic or virulent bacterium—the leucocytes may not be prevented from approaching and ingesting it. When the leucocytes act differently, it is due to the fact that they are repelled by a substance which acts between them and the object to be phagocytosed.

To summarize; in the higher vertebrates and in some lower vertebrates, immunity to anthrax depends upon two factors. In the first instance, upon the fixed phagocytes, represented by the receptive cells of the skin, probably the reticulo-endothelial cells; and in the second instance, upon the motile phagocytes or white blood cells. It is the receptive cells of the skin, in association with the new substance produced by the bacterium, which creates the chemotactic movement. The white cells must respond to this movement; and depending upon the circumstances, are either attracted to or repelled from an infected area.

We have spoken at length about anthrax infection, because in this disease the idea of the receptive cell is brought out with greater clearness than in the other diseases. Here we easily see the part played by the cells in the mechanism of this infection; we can also see, to a certain degree, the part that the cells play in creating the immunity; at least we may form an opinion regarding the relative importance of the receptive cells of the skin and of the white cells.

The skin is not the only organ that contains receptive cells. The intestinal wall contains them also. We are aware of many viruses that act on the intestine in or about the same manner, as the anthrax bacillus does upon the skin.

Because of the technical difficulties, we are prevented from experimenting on the intestinal wall with the ease that is employed in the case of the skin. But an anology between these organs follows, from all the facts gathered, which appears to be well established.

As we have already brought out in the previous chapters, the elective localization of the virus is quite definite. Whether dysentery, typhoid, paratyphoid or cholera virus, be introduced into the circulation, into the peritoneal cavity, or even under the skin, the small intestine finally attracts it. The bacteria are most often found in the lumen of the intestine, while the other organs remain quite free of them. This peculiarity was also recently shown by Sedan and Herrmann in regard to the typhoid bacillus, when inoculated in the ocular conjunctiva.

The reaction, which is the defense of the body against infection, is almost entirely limited to the intestine, irrespective to the portal of entry of the virus. When the resistance of the intestinal wall is decreased, as after

a laboratory artifice, we find that the entire immunity of the animal is interfered with. For after the rabbit had ingested bile, we find that the dose of paratyphoid bacilli necessary to kill is greatly decreased.

On the other hand, by strengthening the intestinal wall after vaccination by the mouth—we refer particularly to acquired immunity—we find that the resistance of the entire animal increases in an appreciable manner.

We should speak then of the receptive cells of the intestinal wall, which in dysentery or typhoid—paratyphoid infections, fulfill the same function as certain receptive cells of the skin possess in anthrax, or in staphylococcus or streptococcus infections.

There exist in the various organs of higher animals, highly differentiated cells which act as local phagocytes. These cells enter into reaction only with certain definite viruses, contrary to the action of the leucocytes or motile phagocytes. The latter attack indiscriminately all foreign bodies; whether they be living or dead.

It is these receptive cells, which are the exclusive property of highly differentiated beings, that have a specific affinity for certain viruses. It is these fixed phagocytes—cells of the reticulo-endothelial layer, intestinal lymphatic cells or other cells—that direct the chemotactic movement of the free phagocytes and assure, with the aid of the latter, the immunity of the entire animal organism.

III

Natura non facit saltus: the passage from natural to acquired immunity should take place in an imperceptible manner. We do not believe that an animal, which has acquired an immunity following a disease, or after an artificial vaccination, is protected in a different manner from a naturally refractory animal. For this reason,

we feel that the receptive cells occupy a place of the greatest importance in the process of actively acquired immunity.

In the chapter on anthrax, we have seen the great importance of the skin in immunity. As long as we attempt to vaccinate the guinea-pig by ordinary methods, we do not succeed. But when we vaccinate the cutaneous tissue, we meet with success; the guinea-pig which resisted all attempts at vaccination, since the discovery of Pasteur, may now be immunized, if we vaccinate the sensitive part—namely, the skin. We made the same observations in regard to staphylococcus and streptococcus infections.

It is then, by strengthening the specific sensitive cells, by accustoming them to the virus, to the exclusion of all other cells, that we have succeeded in obtaining an active immunity.

It is far from our intention to indicate that this is a general law, to which all pathogenic bacteria may be applied. All that we may state is, that we have succeeded by vaccinating the receptive cells, where our predecessors have failed.

It should be noted that the property we ascribe to the cells on being vaccinated, that is, to their accustoming themselves to the virus, has nothing unusual about it. We are aware of many instances of living beings adopting themselves to unaccustomed conditions of life. We know of the facility with which bacteria and infusoria become accustomed to various concentrations of bichloride of mercury—concentrations that are fatal to new strains. It is known that yeasts become accustomed to fluoride solution; as do various bacteria to antiseptics, quinine and to colors. No one denies that the plasmodium may become accustomed to all sorts of toxic substances and

so acquire a real immunity towards them. Even in higher beings this is demonstrated, for it is not rare to find an immunization of a certain group of cells to a toxic substance. An example is the instance of the red cells of the rabbit, which may become immunized against the serum of the eel. If this holds true for red cells, which are easily controlled, why should it be less so for the cells of the skin, of the intestine or of other organs?

It is highly probable that this phenomenon of adaptability, which permits cells to become accustomed to an unsuitable medium, is a frequent or even a general property of living matter.

How do we explain the mechanism of acquired immunity, that follows an attack of an infectious disease? We may imagine that during the course of the disease, the receptive cells are continually brought in contact with the pathogenic bacterium and its derivatives. The reactions between the cells and the virus, lead to a condition of adaptability of the one for the other. The initial sensitiveness of the receptive cells becomes diminished, so that they become indifferent to a new invasion of bacteria. This reaction is so complete that the cells which previously were so prompt to react, now become "accustomed" or "vaccinated." They now act, in the presence of a new infection, exactly as if they were in contact with a saprophyte. The white cells now, not being hindered by a negative chemotaxis, and obeying their voracious nature, attack the newly arrived bacteria, capture them and submit them to the fate of phagocytosis.

Active immunity, which follows a first attack of a disease, depends then according to our notion, first upon the accustoming of the receptive cells which have

become insensitive to the virus, and secondly, to the interference of the phagocytes which can not be prevented from carrying out their function.

A comparison taken from anaphylaxis, or better from antianaphylaxis, may be of assistance in making our idea on the mechanism of vaccination clear. A guinea-pig that has been injected subcutaneously with a small quantity of horse serum shows a sensitization fifteen days later. From this moment on the animal is marked for life. If reinjected with a dose of horse serum that is inoffensive for a normal guinea-pig, the sensitized animal reacts with a fatal shock.

We now know that it is a simple matter to remove the guinea-pig from this deleterious hypersensitivity. All we need to do is to have the animal benefit from the injection of small increasing doses of serum. The anti-anaphylactic state, which follows the anaphylactic state, results as we have shown, upon the *desensitization* of the animal; it finally returns to a normal state.

We can conceive that an animal that is endowed with receptive cells—such as the guinea-pig in regard to anthrax—is sensitive to this virus, because sometime previously its cutaneous tissue was sensitized, and that since then, this hypersensitivness to the anthrax virus was transmitted from guinea-pig to guinea-pig during generations. While practising cutaneous vaccination, exactly as in practising antianaphylactic vaccination, all we do in reality, is to desensitize the cells in regard to the virus. In anthrax, the problem therefore should consist in desensitizing the cutaneous tissue, especially the fixed phagocytes of the skin. By conferring an anthrax immunity on the guinea-pig, all we do is to liberate it from a hypersensitive state, which it has inherited from its ancestors. We bring the guinea-pig

back, in other words, to the natural insensitive state which it previously possessed.

Immunity acquired following an attack of an infectious disease, depends according to our idea, on the desensitization of the receptive cells.

We are far from denying all the importance of antibodies in the genesis of active immunity. What we have tried to show is, that in certain diseases, such as anthrax, streptococcus and staphylococcus infections and a few others, an immunity may be established, and that it may be solid, without depending upon the presence of known antibodies.

We have just described the immunity that follows a spontaneous or experimental attack of a disease.

Are the conditions different in an animal which has been immunized by means of a vaccine? The affection which the latter produces is, most often, imitative to a slight degree of the disease. Also the immunity that follows it is much less solid. This is true even though the mechanism of the immunization is essentially the same in both instances.

In order to make this point clear, we shall take the example of an animal being vaccinated against cholera. To do this, we inject dead bacteria subcutaneously, intraperitoneally, or intravenously.

No matter what the method of vaccination may be, the cholera antigen finally reaches the receptive cells of the intestine. It is there that the first contact occurs between the cells and the virus, that finally leads to a desensitization of the intestinal cells. It surely can not be the bacterial bodies themselves, which are coagulated by heat or chemicals, that enter into reaction with the receptive cells, but it is their soluble derivative or anti-

virus. This has the function of accustoming the receptive cells, of dulling their sensitiveness to the antigen, or in other words of immunizing them.

The liberation of the soluble antivirus is preceded by a disintegration of the bodies of the vibrions; and this is accomplished by the white cells.

As a matter of fact, all entrance of a foreign substance into the body—whether it enter by the subcutaneous, intravenous, or intraperitoneal route—is followed by an influx of white cells at the point of penetration of the virus. After capturing the vibrions, the leucocytes break them up and then digest the stroma of the bacterium. The breaking up of the bacterial bodies results in the liberation of the antivirus; the digestion of the protein stroma is followed by the liberation of substances that we call bacterial cytolysins, that is, agglutinins, precipitins, or other known antibodies.

The phagocytes then are very important in the process of immunization, for they liberate the antivirus to be taken up by the receptive cells on the one hand, and create cytolytic antibodies on the other.

The breaking up of the bacteria by the leucocytes takes a certain period of time. This accounts for the fact that the immunity is not established until after a period of 4 or 5 days. This period may be considerably reduced. It may even be reduced to 24 hours, provided we produce *in vitro* what the leucocytes do *in vivo;* namely if we inject the antivirus, prepared in advance, in the form of filtered cultures.

To summarize; it is the receptive cells or fixed phagocytes of the skin or intestine that are vaccinated. It is the free phagocytes in the blood that render the vaccine assimilable for these cells. Active immunization is therefore brought about by the active coöperation of both of these factors.

In the instance where vaccination is produced by the mouth, the prior autolysis of the bacterial bodies takes place along the intestinal wall. This may be facilitated either by fasting or by a previous preparation of the intestine, such as the ingestion of bile. The immunity produced in this case takes place much more quickly than after vaccination by injections into the body.

What is the function of antibodies?

"The production of antibodies is the essential characteristic of acquired immunity," Bordet tells us.[4] "May we," he asks, "attribute entirely to the presence of antibodies, the superiority of resistance of a vaccinated organism, as compared to a normal animal of the same species? There is no doubt that in a general manner the reply to this question should be in the affirmative." "Does not the injection of the serum of a vaccinated animal to a normal animal transmit its properties to the second animal?" cautiously remarks Bordet.

According to Bordet then, acquired immunity depends essentially upon the presence of antibodies. Bordet raises no questions to this idea. That which appears less certain to him however, was the part that each of these antibodies plays in the problem of defence. He realizes that this problem is difficult for the biologist. He reviews the importance of each type of antibody: agglutinins, complement fixing substance, bacterolysins, etc.

He does not lay great stress on the importance of agglutinins in the struggle against the infection. They may be of a certain importance with a motile virus; for, immobilized by the agglutinins, the bacteria are more easily captured by the phagocytes, than if they retained their

[4] Immunity in Infectious Diseases, p. 92.

motility. In regard to this, Bordet recalls our former experiments with typhoid bacilli, which show that when they are agglutinated with normal ox serum, they become much less toxic. After agglutination, many fatal doses of these bacteria may be tolerated. This is all the assistance that may be expected from the agglutinins.

As for bacterolysins, they may be of use in certain cases. They may also be very dangerous. Pfeiffer himself showed that occasionally they are like a knife with a double edge, because of the liberation of endotoxin in the immunized animal.

The complement fixation antibody, which has made the name of Bordet famous, has its function better defined "When we immunize an animal against a bacterium," Bordet says, "an antibody is produced, which renders the bacterium specifically accessible to the influence of the alexin, and so permits the organism to employ specifically its bactericidal weapon. It is because of this mechanism, because of the association of specific sensitizer and the deleterious alexin, that certain bacteria undergo a marked change which may cause their death." This is the well-known principle of the two substances; this is the principle that is the basis of acquired immunity, both active and passive.

No biologist now denies the general importance of this theory of Bordet, or its fortunate practical applications. We need not emphasize this further.

But does the presence of the complement-fixing body in the serum always correspond to the degree of immunity of the animal? It is present in tubercular subjects, even in large quantities, even though they are rapidly deteriorating toward death. Horses infected with glanders also show it, even though the outcome be fatal. The serum of

rabbits injected with hog-cholera, is rich in antibodies and it may protect guinea-pigs against infection, but the animals show no evidence of active immunity.[5] Bordet admits that we can not measure the preventive or curative value of a serum, only by measuring one of these properties. "We look further," he says, "to see what dose of serum is capable of preventing or stopping an infection." In other words, we decide not to draw any conclusions from the presence of demonstrable antibodies *in vitro*, and turn to the animal, which is tantamount to saying that we return to the unknown.

If the presence of sensitizer or antibodies in general, does not indicate the preventative properties of a serum, then the inverse is also frequent. The patient, whose serum is poor in antibodies is not necessarily one that has a feeble immunity. For example a patient cured of a cholera infection, who, in spite of his poverty in antibodies, resists a cholera infection much better than a subject who is artificially immunized and who has his serum loaded with antibodies. Other examples of the same nature are well known. When injecting horses with cultures of streptococcus by various routes in order to prepare a serum, we have noticed many times, that the titre of antibodies is of less importance in determinning the activity of the serum, than the route of penetration of the virus.

When we exhaust the specific serum by adding the corresponding bacterium, we occasionally see the antibodies disappear, but the protective property remains. Facts of this nature were observed in the case of antibodies against anthrax, chicken cholera and swine erysipelas. Therefore, a specific serum may be derprived of its antibodies without interfering with its activity.

[5] Julius Citron, Zeitschr f. Hyg. u. Infektionskr , Vol. LIII; p. 535, 1906.

Bordet has not missed these troubling facts, and admits that the mode of action of these sera has not been explained, and adds, "The most probable hypothesis is that these sera contain antiaggressive substances (antileucocidins, for example) which permit the production of an exudate which is very rich in leucocytes, and which may have a deleterious effect on the virus—analogous to that which occurs with the virulent streptococcus."

We wish to return to the idea that we have already developed several times in this book. The known antibodies should be without hesitation, stripped of their importance; their function in immunity is, in reality, entirely secondary, or negative in certain cases. However this may be, their function is quite different from what is now thought to be the case.

In animals vaccinated by way of the skin or by the mouth, it is rare to find antibodies in an appreciable amount; most often they are absent in complete immunization. In animals immunized after an infection, these antibodies, on the contrary, are found in abundance. The conclusions drawn from our studies is that the antibodies follow the immunity, but do not precede it. The antibodies appear in the body each time a foreign protein enters it.

Shall we not consider the agglutinins, sensitizer and other known antibodies rather as the cytolysins of the protein stroma of the bacteria? These cytolytic antibodies, we believe, are simply the excretory products, resulting from the intracellular digestion of the stroma, and hence are of secondary importance in the production of active immunity.

What may be said about passive immunity? Is it entirely due to antibodies, as is usually thought?

If we only refer to the infections studied above, we are led to believe that in passive immunity, the antibodies do not act alone in assuring the immunity, but are greatly aided by the antivirus.

We regard the constitution of a specific serum—for example, antistreptococcic or antimeningococcic, and its mode of action, in the following way.

In order to prepare these sera, we know that it is necessary to make a number of injections of cultures of streptococcus or meningococcus subcutaneously, or better still intravenously. Directly after the entrance into the body, the streptococcus or the meningococcus becomes the prey of the phagocytes. The latter rapidly disintegrate the cocci and then digest them. The digestion of the stroma of the bacteria leads to the production of cytolytic antibodies, namely agglutinins, complement fixing body, precipitins and others.

The toxic substance contained in the streptococcus or meningococcus, is unable to produce antibodies. These viruses, properly speaking, are more or less modified by the leucocytes and are then thrown into the circulating blood. These modified viruses, which we call antivirus for the reasons given, are directed soon after liberation, by their affinity, toward their respective cells, and are absorbed by them. After a number of bacterial injections, a certain moment arrives when the affinity of the receptive cell becomes satisfied, and the antivirus is not tempted to join them any more. The antivirus then remains in the blood, where it circulates freely, in association with the antibodies of the stroma; that is, in association with the agglutinins, complement fixing body and others. The specific serum then contains the antivirus, which was previously described as occurring during the course of active vaccination, and the antibodies.

What happens when we confer passive immunity to an animal? The antivirus contained in the serum is directed to the receptive cells in the animal and vaccinates them. The antibodies, which continue to circulate in the blood, only become active at the moment when the animal is infected. The antibodies in this instance sensitize the virus, streptococcic or meningococcic, and so render them more apt to become phagocytosed.

We easily explain the facts that have been brought out above, in the light of this hypothesis. They are inexplainable by the present theory. By virtue of this hypothesis, all the manifestations of immunity, whether natural or acquired, active or passive, are regarded as one process—that is, the interaction of the antivirus with the receptive cell.

If it is true, as we have just stated, that all the manifestations of immunity depend on the same principle, how then can we explain the difference between active and passive immunization?

We know that the immunity which follows active immunization is long and demands a period of preparation of at least several days. The immunity following passive immunization is, on the other hand, of short duration and is rapidly established.

These differences, which appear complex, are in reality simple. An animal that has been injected with vaccine, can only employ the antivirus, after the bacterial bodies are disintegrated. This process demands a certain period of time. The preparatory work is spared the animal which receives with the specific serum the antivirus already prepared.[6]

[6] The sensitized bacterial vaccines unite, as we know, the advantages of active and passive immunization. The immunity appears promptly

The other characteristic in which the two types of immunity differ, is in their duration. This difference is explained in the same way. According to our hypothesis, the immunity lasts as long as the antivirus remains present in the receptive cells.

The antivirus which comes from the vaccine, that is from bacterial bodies, acts *in statu nascendi*, which is not the case for that which is present in an antibacterial serum. The antivirus coming from the vaccine, acts in a state of greater concentration, and further it may remain in the body for a longer period of time. This is not what happens with the antivirus, which is carried in the antibacterial serum, that is to say, by a foreign albumin. The effect of the antivirus derived from the vaccine lasts longer, because it is not all liberated at once, the disintegration of the bacterial bodies requires a certain period of time.

These differential characteristics, pertaining to the action and the duration of the reaction between the receptive cells and antivirus, are particularly increased when the immunization is conducted with living virus.

and remains for a long time. It was believed until recently, that the persistence of the immunity was due to the bacterial bodies, and that the rapidity of its appearance was due to the presence of the sensitizer In other words, the animal vaccinated with the sensitized virus is benefited in the first few days by passive immunity, which is then followed by an active immunity.

This interpretation, which we held also, now appears incorrect. We are rather led to believe that the sensitization of the virus leads to the beginning of the digestion. This decreases the work of the phagocytes and accelerates the liberation of antivirus. The immunity conferred by the sensitized vaccine, is not therefore passive to begin with and active thereafter. From the very beginning, it is the same as that conferred by an ordinary vaccine, with this difference; the disintegration by the leucocytes being more rapid, the liberation of antivirus requires a shorter period of time.

In this case, the persistance of the antivirus in the body is particularly long; it may even be unlimited in time. We are aware of the long refractory state of an individual, after an attack of an infectious disease. The disease may have disappeared for some time, and still the individual continues to benefit from a solid immunity.

How do we conciliate this fact with our hypothesis?

Should not the antivirus completely disappear from the circulation, considering that its source has disappeared with the termination of the disease? Does the production of antivirus really cease with the termination of the disease? When the clinical symptoms have disappeared, it appears that no trace of the virus is present. But the remains of the virus may be hidden in some part of the body and so feed it with antivirus, which keeps up the immunity. This is only a supposition, and it may be regarded a bold one. But the audacity of this hypothesis may be lessened, by citing a few facts.

We recognize certain bacteria, such as the typhoid bacillus, the staphylococcus, and the tubercle bacillus which may be present in the body for years, without interfering with the health of their host. At some time, because of a traumatism, an intercurrent infection, or some undetermined disturbance, a typhoid osteitis or a staphylococcus osteomyelitis occurs. This is the remains of an old infection which was not detected.

In the cases referred to, it is only by chance that the presence of the virus is detected in the body. But we have instances where the virus is not hidden and where it continues to circulate in the blood, in spite of the immunity acquired by the animal; this is observed during an infection with piroplasmas. We believe that cases of latent microbism are much more frequent than is generally believed. We are also led to believe, that the

long period of immunity, which characterize certain infectious diseases, are in many instances due to the living bacteria which survive the disease and which contribute to an uninterrupted production of antivirus.[7]

To summarize: The immunity, active or passive, is due to one single mechanism and is not in opposition to the fact that it may appear rapid or may vary in duration.[8]

What may be said about vaccinotherapy in this relation?

[7] Let us recall that in the experiments made on hog cholera by Julius Citron, he found that animals, which were actively vaccinated and were capable of conferring a passive immunity, still conserved bacteria in a living state for a period of six months. (Zeitschr. f. Hygiene und Infektionskrank, Vol. LIII, p. 515, 1906.)

[8] That which has just been said regarding immunity applies equally to anaphylaxis

It is ordinarily believed that the anaphylactic state is due to the presence of antibodies. When horse serum is injected into a guinea-pig we wait 12 to 15 days for the anaphylactic state to become established. This delay is explained on the basis that the antibodies are being formed. When a guinea-pig is injected with rabbit antihorse serum, we create an anaphylaxis at once, which is called passive; this too, is supposed to be due to the presence of antibodies which are present in the serum.

We should ask whether the antibodies actually take part in this instance· Is not the sensitizing substance the same in active and passive anaphylaxis? We do not know its nature at present, but the little that we do know about it, leads us to believe that it already exists in the horse serum and that its action is masked by another substance which is also present in the same serum (anti-anaphylactic substance). In order that the sensitizing substance may show its effect, it is necessary that the other—the anti-anaphylactic substance—should be eliminated at first. It is for this reason that it is necessary to wait 12 to 15 days in the case of active anaphylaxis.

It is also for this reason that anti-horse rabbit serum acts so rapidly. The anti-anaphylactic substance—which is probably associated with the protein of the horse—is already eliminated during the course of preparation of the rabbit. Whether we are dealing with active or passive anaphylaxis, according to this hypothesis, we are dealing with the same substance, which is not an antibody.

Because of the method of action of "curative immunization," vaccinotherapy occupies an intermediary place between active and passive immunization.

Now that we have described the mechanism of one and the other, what we think about vaccinotherapy may be easily surmised.

According to Wright, who was the creator of this method, it is the healthy areas of the organism that are called upon to help the infected regions. This assistance depends upon the production of antibodies. In order to produce these antibodies Wright injects bacterial bodies by subcutaneous or intravenous inoculation. The doctrine of the English scientist is so well known that we may pass over the details here.

As we have already indicated, in dealing with the subject of the treatment of staphylococcus and steptococcus infections, vaccinotherapy according to our idea, aims not at the cure, but at the limitation of the area of infection. It is the non-infected receptive cells in the neighborhood of the infected cells that should be aimed at in vaccinotherapy.

As the object of vaccinotherapy is the protection of the healthy cells, it should render them incapable of contamination from their sick neighbors. In order to bring about this prophylaxis, the antivirus must be brought in contact with the receptive cells. The antivirus exerts a double action: it interfers with the spread of the virus locally, and by coming in contact with the healthy receptive cells, and being absorbed by them, it renders the cells invulnerable to the virus.

We can make this process clear by citing an experiment:

An agglutinating serum is heated to 75°. It loses its agglutinating power. If we bring it in contact with

the bacteria they are not agglutinated. These non-agglutinable bacteria, subsequently brought in contact with an agglutinating serum, will not be agglutinated. What has happened? The bacteria, even though they have undergone no apparent change, have become resistant to the agglutinin.

We believe that an analogous phenomenon takes place at the site of the receptive cells of the skin or of the intestinal wall, or of any other organ. The contact brought about by the presence of the antivirus, is sufficient to immunize the receptive cells. Following this the cells may be brought in contact with virulent bacteria, and they react exactly as if the bacteria were inocuous. The affinity of the cells being exhausted, they cease momentarily to be receptive.[9]

Vaccinotherapy as we conceive it, does not differ in any way as to its mechanism, from active immunization. It reacts with the healthy receptive cells; its action is entirely prophylactic.

It should be noted in passing, that this prophylactic character is even found in the most active sera—such as in antidiphtheritic and antitetanic sera. These sera also act by interfering with the fixation of the toxins to the cells, rather than by curing the already diseased cells.

Whatever the method employed be, in order to obtain immunity—whether it be active vaccination or vaccinotherapy—the principle of immunization is always the same. It rests on the protection of the receptive cells, that is the first cells which are attacked by the virus,

[9] It may be recalled that this conception of exhaustion of the affinity of the cells approaches the conception formulated by Pasteur, who attributed the immunity of the chickens vaccinated against cholera, to the exhaustion of the nutritive properties in the blood.

the fixed or local phagocytes, at the site where the active part of the infection occurs.

We are led to believe that the same process operates with certain antibacterial sera. It is possible that the good effects of antimeningococcus, antistreptococcus or other sera, are due less to the action of known antibodies, than to the antivirus which acts in the manner already described.[10]

We know since the time of Bichat, that each organ is endowed with certain groups of cells with special characteristics, as far as anatomical structure and physiological function are concerned. The facts now indicate that this differentiation of the cells extends to the domain of infection and immunity. Have we not shown, during the course of these researches, that certain groups of cells are alone susceptible to infection and immunization, without the entire animal organism taking part in it?

We have seen that the skin is much more than a simple "sheath protector" in regard to certain viruses.[11] It has been noted that the same holds true for the intestinal wall, which may fulfill an active function, in infection as well as in the defense. We therefore come to the conclusion that every virus has its cell, and every cell its immunity.

Inspired by these ideas regarding the mechanism of infection and immunization, we endeavored to obtain better results by employing the preparations already known. The most solid immunity is that which is con-

[10] An interesting fact in regard to anti-anthrax serum was recently stated by Matzumato This serum injected *under the skin* in a dose of 0.25 cc. protects the rabbit against death, while 2 cc. injected *in the vein*, that is, a quantity 5 times as much, results in a failure of the serum to prevent a fatal anthrax septicemia. (Zeitschr. f. Immunitatsf , Vol. XL, July 30, 1924, p. 425.)

[11] E. Metchnikoff. Immunite dans les maladies infectieuses, p. 423.

ferred by a natural infection. The plan in artificial vaccination therefore is to follow the routes which the virus takes in its penetration into the body. Immunization by the cutaneous or oral route, conceived in this work, is nothing more than an application of the old principle: *natura non vincitur nisi parendo.*

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